PHYSIOLOGY OF SUSTAINING WHEAT YIELD UNDER LATE PLANTING HEAT STRESSED ENVIRONMENT

MD. ABU HASAN

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BANGABANDHU SHEIKH MUJIBUR RAHMAN AGRICULTURAL UNIVERSITY, GAZIPUR-1706, BANGLADESH

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MD. ABU HASAN

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MD. ABU HASAN

Registration No. 2001-05-951

Certificate of Approval:

Prof. Dr. Jalal Uddin Ahmed
Major Professor and Chairman
Advisory committee

Prof. Dr. Tofazzal Hossain
Member
Advisory committee

Prof. Dr. M. A. Khaleque Mian
Member
Advisory committee

Prof. Dr. M. Moynul Haque
Member
Advisory committee

Dedicated

to

My beloved parents,

wife and children

BIOGRAPHICAL SKETCH

The author was born on January 1971 at Nilphamari district of Bangladesh. He is the first of three children of Mr. Md. Abdus Samad and Mrs. Hasina Begum.

The author received primary education at Nilphamari Government Primary School, Nilphamari. He completed his secondary school education from Nilphamari Government Boys High School and higher secondary education from Nilphamari Government College, Nilphamari and obtained first division in both the examination in 1986 and 1988, respectively. He completed B. Sc. Ag. (Hons.) degree in 1992 (held in 1996) with first class from Hajee Mohammad Danesh Agricultural College, an affiliated institution of Bangladesh Agricultural University, Mymensingh. He obtained M. S. degree in Crop Botany in 2002 from Bangabandhu Sheikh Mujibur Rahman Agricultural University with CGPA 4.00 out of 4.00.

The author started his professional career in 1997 as a Lecturer at Patuakhali Agricultural College, Patuakhali. Now he is working as Associate Professor in the Department of Crop Physiology and Ecology at Hajee Mohammad Danesh Science and Technology University, Dinajpur.

He is the member of Krishibid Institution, Bangladesh Botanical Society and Asiatic Society of Bangladesh.

He is happily married to Rehana Banu and they are blessed with a son (Ridwan Hasan) and a daughter (Rumaisha Hasan).

Md. Abu Hasan

DISSERTATION ABSTRACT

PHYSIOLOGY OF SUSTAINING WHEAT YIELD UNDER LATE PLANTING HEAT STRESSED ENVIRONMENT

By

Md. Abu Hasan

Development of heat tolerant wheat genotypes and/or amelioration of the effect of heat stress through agronomic strategies are the possible options to sustain late planting yield reduction of wheat in Bangladesh. In the present investigation, three laboratory and two field experiments were carried out during July 2006 to October 2008. Twenty wheat genotypes were tested in laboratory experiment to investigate seed reserve utilization efficiency (SRUE) during germination and proline level at seedling stage as screening criteria against heat stress. Based on membrane thermostability test, the wheat genotypes showing <50% membrane injury were grouped as heat tolerant (HT) and the wheat genotypes showing ≥50% membrane injury were classified as heat sensitive (HS) genotypes. The HT wheat genotypes maintained higher (1.042 g/g) seed reserve utilization efficiency than that of HS genotypes (0.682 g/g) at high germination temperature but none of them showed such variation under optimum germination temperature. At high growing temperature (35°C) the HT genotypes produced more than double (>200%) proline than that at 25°C but the HS genotypes produced less quantity of proline at 35°C compared to that in HT genotypes. A significant negative correlation was found across the wheat genotypes between membrane injury (%) and SRUE at 35°C ($r = -0.78^{**}$) and also between seedling proline content at 35°C and percent membrane injury ($r = -0.619^{**}$).

Out of twenty genotypes, four heat tolerant (Kanchan, Fang 60, BAW 1059 and BL 1022) and two heat sensitive (Pavon 76 and Sonora) wheat genotypes were included

for the field evaluation of physiological sensitivity and grain yield in heat stressed environment. Unlike HS genotypes a remarkable increase in kernel proline level due to heat stress was found in HT genotypes during early stages of rapid grain growth period which was found in flag leaf during later stages of rapid grain growth period. Flag leaf longevity reduced in all wheat genotypes due to late planting heat stress but the reduction was inconsistent between HT and HS wheat genotypes. The HT wheat genotypes showed longer grain growth duration, longer duration of rapid grain growth rate, higher stem reserve utilization and consequently maintained lesser reduction in grain dry weight per ear. Thus the relative grain yield of HT genotypes was higher than that showed by HS wheat genotypes under heat stress condition. Flag leaf and kernel soluble sugar level revealed that irrespective of heat tolerance Kanchan, Fang 60, Pavon 76 & Sonora were found to be sink limiting for kernel development under heat stress condition. Among these four genotypes sink limitation appeared 4 to 8 days later in HT genotypes than HS ones. In other two HT genotypes (BAW 1059 and BL 1022), sink limitation did not appear clearly under heat stress condition.

Application of additional nitrogen and/or splitting of recommended dose of nitrogen fertilizer (RDNF) after anthesis was evaluated in the field in late planted wheat. Three HT (Kanchan, Fang 60 and BAW 1059) and one heat sensitive (Sonora) wheat genotypes were tested in this experiment. Three splits application of 50% extra N fertilizer + RDNF showed increased grain yield compared to that obtained from RDNF under heat stress conditions only in sink non-limiting genotype, BAW 1059. Longer flag leaf activity and greater stem reserve utilization mainly contributed to this yield increase in sink non-limiting genotype (BAW 1059).

The effect of parent plant growth temperature was evaluated in terms of physiological attributes contributing to seed vigour. Seed vigour (as indicated by seed density, seed

conductivity, seedling dry weight, production of normal seedling, seed reserve utilization efficiency and % seedling emergence) was more adversely affected in heat sensitive than the heat tolerant wheat genotypes due to higher parent plant growth temperature.

The overall results indicate that determination of heat tolerance in wheat genotypes either by increased proline level or by increased seed reserve utilization efficiency, as found in the present study, were as effective as the widely used cell membrane thermostability test. However, for sustaining wheat yield under late planting heat stress condition additional nitrogen fertilizer was found to be effective only in sink non-limiting wheat genotype.

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Autumn 2009 The Author

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CHAPTER I

GENERAL INTRODUCTION

Wheat (*Triticum aestivum* L.) belongs to the grass family *Graminae* and to the tribe *Hordeae*, forming a true spike. The species of *Triticum* are grouped into three ploidy classes: diploid, tetraploid and hexaploid. The hexaploid species, *Triticum aestivum* is the principal wheat in commerce. Based on vernalization requirement *Triticum aestivum* is of two kinds- spring or winter type. The spring type cultivars grown in subtropical climates are normally insensitive to photoperiod.

Wheat is the most widely grown cereal in temperate environments, and is also cultivated in many tropical cropping systems, where it is often grown as the winter season crop in rotation with a number of other crops. Wheat is an important cereal crop and ranks first globally and second in Bangladesh both in terms of production and acreage (Anonymous, 2005b). It covers about 0.52 million hectares of land in Bangladesh and produces about 1.03 million tons per year with an average yield of 2.0 t/ha (an average of 2002-2003 to 2007-2008) (Anonymous, 2005b, Anonymous, 2007 and Anonymous, 2008). In Bangladesh, about 60% of the wheat is cultivated at late sowing condition after harvesting the transplanted aman rice (Badaruddin *et al.*, 1994). Late planting wheat exposed to high temperature (mean air temperature of >26°C) at reproductive stage causing reduction in yield and it is one of the major reasons of yield gap (2 t ha^{-1}) (Islam *et al.*, 1993; Hasan and Ahmed, 2005).

However, this problem will be further increased due to global warming. Because in Bangladesh also the increase in temperature would be 1.3°C and 2.6°C by the year 2030 and 2075, respectively with respect to the base year 1990. The seasonal variation of temperature will be more in winter than in summer: 1.3°C in winter and

0.7°C in summer for 2030 and 2.1°C in winter and 1.7°C in summer for 2075 (Ahmed and Alam, 1999). Such global warming will push the wheat farming further into heat stressed environment in future and may cause further reduction of present yield level. In spite of low yield of wheat due to post anthesis heat stress, cultivation of wheat can not be avoided totally. Because the irrigation dependent Boro rice cultivation may need to be replaced in future by partially irrigated or non irrigated wheat cultivation to overcome arsenic problem. Therefore, effort ought to be made to minimize the late sown yield reduction by screening or developing high temperature tolerant wheat genotypes/ varieties or by ameliorating the effect of heat stress through agronomic strategies.

Membrane thermostability (MT) test is a widely used and acceptable method to evaluate heat tolerance and heat susceptibility index which is also used to evaluate yield parameters. But they are time consuming, laborious and not suitable for screening a good number of genotypes at a time. The seed reserve utilization efficiency of different crops may vary in different temperature regimes (Gangadhar Rao and Sinha, 1993; Penning de Vires *et al.*, 1979) and the magnitude of variation was different in different genotypes (Gophane *et al.*, 2005; Sikder *et al.*, 2004)). Several studies in wheat found a relation between heat tolerance and seed reserve utilization efficiency (Blum and Sinmena, 1994; Cargnin *et al.*, 2006; Hasan *et al.*, 2004). But the heat tolerance of wheat in relation to seed reserve utilization efficiency was not yet evaluated based on membrane thermostability test.

Under supra-optimal temperature, free proline content is known to accumulate in barley and radish leaves (Chu *et al.*, 1974), in the leaves of Brassica vegetables (Takeda, 1999), in cabbage and Chinese cabbage (Hossain *et al.*, 1995), in apple (Park *et al.*, 2001) and in flag leaves of wheat (Hasan *et al.*, 2007). Under supra-optimal

temperature genotypic difference in proline accumulation pattern has also been reported in six cotton cultivars (Ronde *et al.*, 2001) and in different cabbage and Chinese cabbage varieties (Hossain *et al.*, 1995). Hasan *et al.* (2007) reported genotypic difference in flag leaf and kernel proline level in heat tolerant and heat sensitive wheat genotypes due to post anthesis heat stress condition. But in wheat, the heat tolerance in relation to seedling proline content was not yet evaluated.

Post anthesis heat stressed environments in wheat induces several physiological disorders which eventually results in early onset of senescence or retards conversion of sucrose to starch in developing grains of wheat. Thus, altered source activity or sink activity or combine effect of both due to heat stress may be the casual factors for a yield penalty through reduction in grain filling period as well as the rate of grain filling (Sofield, 1977; Hasan and Ahmed, 2005). But in Bangladesh, wheat improvement program is still running based on late seeding stay green method considering only source is a limiting factor. However, investigation about the physiological sensitivity in relation to source and sink activity would be helpful to take proper crop management to sustain yield under late planting heat stress condition.

Late planting wheat exposed to terminal heat stress, which coincides with grain development and maturation. Evidence consistently shows that exposure of the parent plant to high temperature affects the quality of seed produced. High temperature following anthesis adversely affects grain development in wheat (Tashiro and Wardlaw, 1990a; Hasan and Ahmed, 2005). As a consequence, smaller and shrunken grain is produced at high temperature. Other physical properties of the seed including seed dimensions, seed coat and the final appearance of the seed are also affected by high parent plant growth temperature (Tashiro and Wardlaw, 1990a). Grain

development at elevated temperature can affect membrane integrity and can cause increase in membrane leakage of both electrolytes and macro-molecules during germination which subsequently impair germination and seedling vigor (Girelberg *et al.*, 1984). Grass and Burris (1995a) reported impair germination and decline in seed vigor in wheat reflected in reduced shoot and root dry weight and higher seed conductivity due to high temperature during seed development and maturation. Utilization of the seed obtained from late seeding wheat may cause further reduction in yield. The effect of heat stress on wheat yield and yield components is well documented. But seed quality was often ignored in these studies. So, the physiological quality of wheat seed in relation to heat stress should be evaluated.

For optimal plant growth at above optimum temperature the demand of resources is higher due to increased rate of metabolism. Nitrogen contents in leaves reduces by 10% when wheat plants experience heat stress at the double ridge stage and by 25% at anthesis stage (Woolenweber *et al.*, 2003). Additional amount of N fertilizer may be needed to sustain late planting yield reduction due to heat stress. Application of extra nitrogen or splitting of N fertilizer after anthesis may provide some benefits either by lengthening the leaf activity and/or by increasing stem reserve which might be utilized later for grain development. Therefore the objective of the present study was to sustain wheat yield under heat stressed environments through investigating -

1. Seed reserve utilization efficiency during germination and proline content of seedlings as screening criteria,
2. The sensitivity of physiological traits resulting reduced grain yield in heat stressed environment,
3. The quality of seeds influenced by late planting heat stressed environment and
4. Different nitrogen management practices to sustain wheat yield under late planting heat stressed environment.

CHAPTER II

REVIEW OF LITERATURE

2.1 . Geographical origin of wheat

Wheat evolved from wild grasses, probably somewhere in the Near East, its geographical centre of origin. A very likely place of origin was the area known in early historical times as the Fertile Crescent- a rich soil region in the upper reaches of the Tigris-Euphrates drainage basin. Many wild species of the genus *Triticum* including *Triticum speltoides, Triticum monococcum, Triticum comosum, Triticum umbellulatum, Triticum tauschii, Triticum dichasian, Triticum dicoccoides, Triticum araraticum, Triticum ventricosum, Triticum triunciale, Triticum ovatum, Triticum kotschyi, Triticum cylindricum, Triticum syriacum, Triticum juvenile, Triticum triaristatum etc.* may be found growing today in Lebanon, Syria, Northern Israel, Iraq and eastern Turkey. While several species of wheat were cultivated during early agriculture, in modern agriculture only common wheat (*Triticum aestivum* L.) and durum wheat (*Triticum turgidum* L) are important (Poehlman and Sleper, 1995).

2.2. Cytogenetic origin of wheat

Wheat belongs to the grass family *Graminae* and to the tribe *Hordeae*, forming a true spike. The species of *Triticum* are grouped into three ploidy classes: Diploid (2n = 2x = 14), tetraploid (2n = 4x = 28) and hexaploid (2n = 6x = 42). Feldman and Sears (1981) listed 13 diploid, 12 tetraploid and 5 hexaploid species of *Triticum*. Only two species of *Triticum* are commercially impotant: the hexaploid species, *Triticum aestivum,* the bread wheat- the principal wheat in commerce and the tetraploid species, *Triticum turgidum,* the durum wheat that is used in making pasta. The wild

tetraploid species, *Triticum timopheevii*, is the source of cytoplasmic male sterility utilized in breeding hybrid wheat.

On the evidence of current archaeological findings, tetraploid wheat, *Triticum dicoccum* was probably the first cultivated wheat, but may have arisen in the wild and become established as a result of husbandry and artificial selection of naturally occurring stands of wild wheat. The other tetraploids have evolved from the initial cultivated form by selection of a series of modifications to the current free-threshing non-fragile forms, such as the widely cultivated *Triticum durum.* The cultivated AAGG tetraploid, *Triticum timopheevii* must have arisen from *Triticum araraticum* in a similar way to that by which *Triticum dicoccum* arose from *Triticum dicoccoides. Triticum militinae*, which arose as a single plant in a population of *Triticum timopheevii*, may be a mutant with free-threshing qualities. *Triticum zhukovskyi* was isolated from mixed cultivated populations of *Triticum monococcum* and *Triticum timopheevii,* and is certainly an amphiploid of these two species (Upadhya and Swaminathan, 1963, 1965). The AABBDD hexaploids are the result of amphiploidy between an AABB tetraploid and the D genome diploid, *Triticum tauschii* (Schmidt, 1974). The hexaploids are all cultivated wheats- no wild forms exist. It is therefore likely that they arose in cultivation. The principal differences between the hexaploid forms are due to single gene, with the possible exception that *Triticum vavilovi* may differ by two genes (Singh *et al.*, 1957; Okamoto, 1962; Sears, 1947).

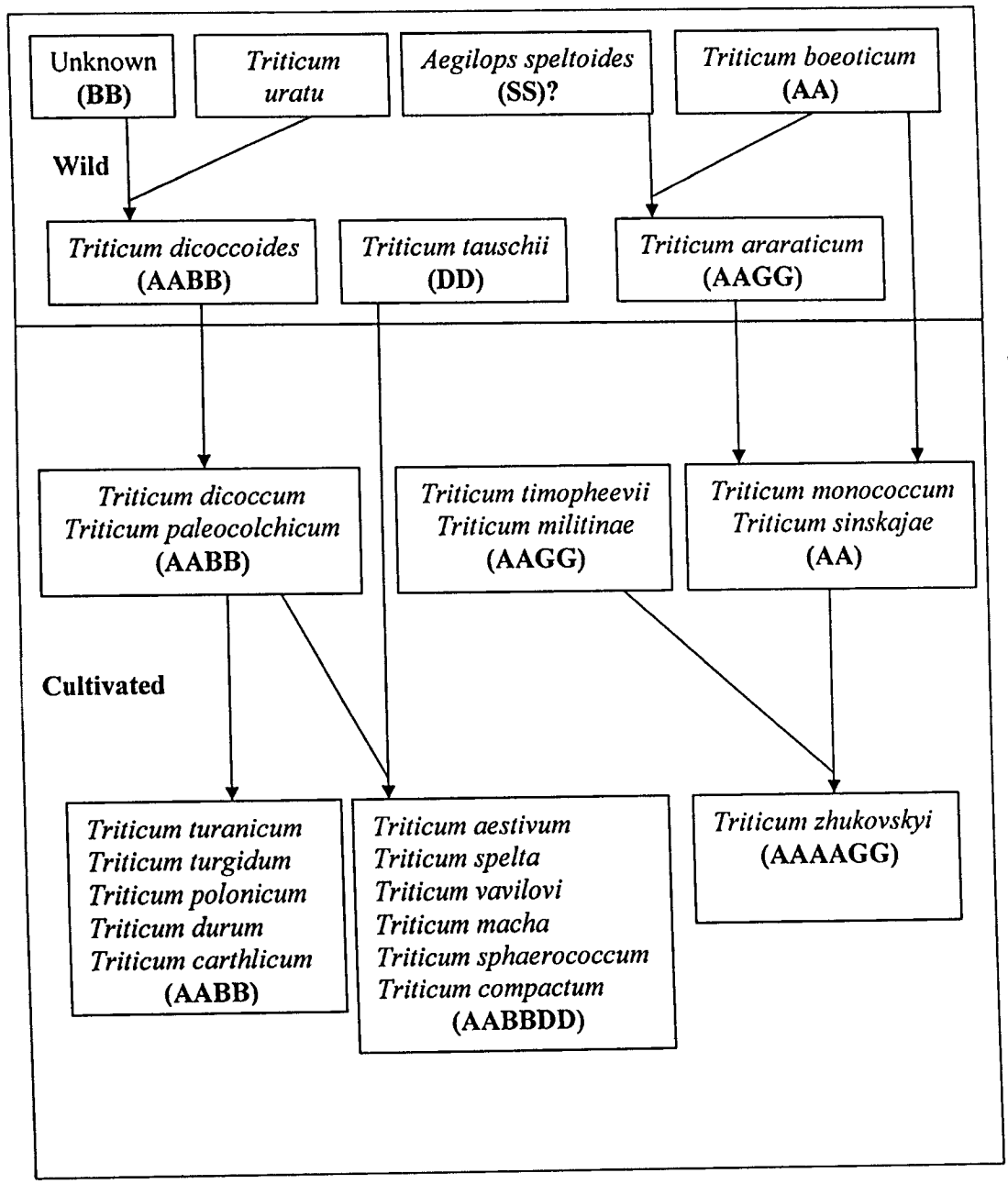


Fig. 2.1. Origin of cultivated tetraploid and hexaploid wheat. (Miller, 1987)

2.3. Winter and spring wheat

The physiological characteristics of the wheat plant that adapts different wheat cultivars to different climates are generally related to vernalization requirement, winter hardiness or cold tolerance and photoperiod response. The vernalization requirement determines the wheat growth habit, whether spring or winter type, and is

a major plant characteristic in determining the kind of wheat is grown in particular regions of the world. Wheat with winter growth habit may be distinguished from wheat with spring growth habit because a period of vernalization (exposure in the seedling stage to near-freezing temperatures) is required before flowering will occur. Another characteristic of winter type wheat that distinguishes them from spring wheat is their ability to harden and withstand freezing temperatures. Differences between winter and spring types are not always distinct as some facultative winter type cultivars have a low vernalization requirement and low tolerance to freezing stress and differ only slightly from spring types. Wheat is grown predominantly in the northern hemisphere, roughly between 25 and 60° North latitude. Winter type wheat are grown where winter temperatures are sufficiently low to meet vernalization requirements, yet not so low that the wheat will not survive. Spring type wheat is planted in the spring and summer seasons north of the winter wheat areas where winter temperatures are too cold for the winter wheat to survive. The spring type cultivars grown in these northern areas are photoperiod sensitive and flower during declining day lengths. The spring type cultivars grown in subtropical climates are normally insensitive to photoperiod and may be grown in any season of the year (Poehlman and Sleper, 1995).

2.4. High temperature stress in wheat

Wheat is the most widely grown cereal in temperate environments, and is also cultivated in many tropical systems, where it is often grown as the winter season crop in rotation with a number of other crops for example, with maize in Africa, rice in Asia, and soybean in Latin America. Among the disadvantages linked to growing wheat in tropical areas; different types of high temperature stress that affect the crop. Continual heat stress affects approximately 7 million ha of wheat in developing

countries, while terminal heat stress is a problem in 40% of the temperate environments, which cover 36 million ha. Continual heat stress is defined by a mean daily temperature of over 17.5°C in the coolest month of the season and over 50 countries (importing more than 20 million tons of wheat per year experience this type of stress throughout the wheat cycle (Fischer and Byerlee, 1991). Temperature requirement of wheat varied depending on growth stages. The optimum temperature for wheat crop growth is about 20°C (Al-Khatib and Paulsen, 1984) and prolonged exposure to temperature exceeding 35°C is not tolerable (Gusta and Chen, 1987; Blum, 1988). Temperature in the range of 20 to 25°C has been considered favorable for wheat seed germination, seedling emergence and optimum plant establishment (Behl *et al.*, 1993). Mean temperature of 16 to 20°C is favorable for tillering (Behl *et al.*, 1993) and greater than 25°C temperature might depress the rate of leaf appearance (Kirby and Perry, 1987). Photosynthesis in wheat is maximal between 22 to 25°C (Blum, 1986; Kobza and Edwards, 1987) and decreases sharply above 35°C (Blum, 1986; Rawson, 1986). The optimum temperature for reproductive stage of wheat lies a range of 22 to 26°C (Campbell and Read, 1968). Temperature above 26°C at reproductive growth phase causes harmful premature ripening of wheat (Aborl *et al.*, 1991). Tashiro and Wardlaw (1989) found a reduction in grain growth duration and dry matter accumulation in grain in wheat with increasing temperature above a mean of 26.7°C. Temperatures above 25°C adversely affect flux through the pathway of starch synthesis in developing wheat and limit yield (Keeling *et al.*, 1994).

2.5. Membrane thermostability

Conductometric measurement of solute leakage from cells was used in several studies to estimate heat damage to the plasma membranes. Genetic variation in membrane thermostability (MT) has been inferred using conductometric measurements in

various field-grown crops, including spring wheat (Blum and Ebercon, 1981). Shanahan *et al.* (1990b) obtained a significant increase in yield of spring wheat in hot locations by selection of membrane-thermostable lines, as determined by measurements on flag leaves at anthesis. By applying the MT test on winter wheat seedlings, Saadalla *et al.* (1990b) found a high correlation in MT between seedlings and flag leaves at anthesis for genotypes grown under controlled environmental conditions. Measurements of MT of 16 spring wheat cultivars were compared internationally with performance at several heat-stressed locations. Variation in MT of both field acclimated flag leaves and seedlings grown in controlled conditions was associated with heat tolerance in warm wheat-growing regions (Reynolds *et al.*, 1994). Other studies have confirmed genetic variation of these materials and indicated high heritability for the trait (Fokar *et al.*, 1998a). Although the physiological basis for the association of MT with heat tolerance has not been determined, plasma membranes are known to be more heat tolerant than the photosynthetic thylakoid membranes, for example (Berry and Bjorkman, 1980). While loss of membrane integrity may be the cause of ion leakage from the cell, this phenomenon could also be caused by thermally induced inhibition of membrane-bound enzymes responsible for maintaining chemical gradients in the cell.

At CIMMYT experiments were conducted on 16 lines of the IHSGE using both seedlings and flag leaves (Reynolds *et al.*, 1994). The MT trait was favorably correlated with yield in a number of heat stressed international environments, using both methodologies. When comparing MT for seedlings versus field-grown flag leaves, there was a significant positive correlation ($r^2 = 0.67$, $n = 16$) indicating that the MT determined at the two development stages was well associated. These data support the idea that using seedlings raised under artificial conditions for screening

MT may be a viable alternative to using field-grown tissue. The use of seedlings is preferable logistically because the conditions of plant acclimation can be controlled, which is not possible in the field. The importance of this point is illustrated indirectly by the data. Using the seedling procedure, the three repetitions of the experiment were measured for MT on three subsequent days. While the interaction of genotype with repetition was not significant, the main effect of repetition (i.e., day of experiment) was highly significant. Even under controlled conditions, unintentional discrepancies either in procedure or day-to-day variability of conditions influenced absolute values of MT. Since it would not be practical for a breeding program to assess MT on all the germplasm of interest in one experimental run, a methodology involving controlled conditions would seem preferable. Another advantage of using seedlings rather than more mature tissue is that MT is unlikely to be affected by phenology at such an early stage of development. In these experiments there was a range in anthesis and maturity dates among the genotypes. Instead of measuring MT on the precise date of anthesis for each genotype, MT values were measured on all flag leaves on the same calendar date and subsequently adjusted using the number of days between measurement of MT and anthesis as a covariate (Reynolds *et al.*, 2001). Heat tolerant genotypes exhibited lower accumulation malodialehyde and H_2O_2 due to increased activities of superoxide dismutase, peroxidase and catalase under high temperature conditions. The higher water retention capacity and lower membrane injury enhanced heat tolerance in wheat (Sunita and Gupta, 2005).

2.6. Seed reserve utilization during germination in relation to heat tolerance

The role of temperature in the germination of seeds is well recognized (Hillel, 1972). Temperature is a modifying factor in germination since it can influence the rate of water supply and other substrates necessary for growth (Wanjura and Buxtor, 1972).

Germination temperature influences the root and shoots growth and dry matter partitioning. A change in the partitioning of dry matter was clearly seen in the decreasing root to shoot ratio of Glycine as day/night temperature was increased from 15/10°C to 36/31°C (Kokubun and Wardlaw, 1998). Gangadhar Rao and Sinha (1993) found at low temperature the proportion of dry matter in shoot and root was almost similar in Sorghum, but at high temperature, dry matter accumulation was more in shoot than in roots.

Seed reserve utilization efficiency (SRUE) was defined as the ratio of gain in seedling (shoot and radicle) DW to loss in seed DW as respiration during germination. Germination temperature influences the efficiency of seed reserve utilization in different plants. The seed metabolic efficiency showed substantial improvement in sorghum at higher temperature of 30°C. At 40°C, there was not much improvement in the seed metabolic efficiency (Gangadhar Rao and Sinha, 1993). 30°C temperature is suitable for higher seed metabolic efficiency during germination of sorghum. Both at 20 and 40°C the efficiency decreased in all genotypes but there is a genotypic difference in seed metabolic efficiency at different temperature regimes (Gophane *et al.*, 2005). Penning de Vires *et al.* (1979) found that above a temperature of 25°C in wheat and 30°C in maize, there was a decrease in the ratio of growth to maintenance respiration and at high temperature the efficiency of respiration in terms of dry matter production was significantly decreased.

Seed endosperm utilization efficiency (EUE) was measured by periodic sampling of germinating seed in the dark under normal (25°C) and high (35°C) temperatures in 18 wheat cultivars by Blum and Sinmena (1994). Heat tolerance of the autotrophic juvenile plant was measured in the same cultivars in terms of plant growth rate from

17 to 24 d after emergence under normal (15 degrees /25 degrees) and high (25 degrees /35 degrees) (night/day) temperatures. Averaged over 18 cultivars, EUE was reduced by chronic heat stress from 0.64 g g-1 at 25 degrees to 0.53 g g-1 at 35 degrees. Percentage growth rate reduction from 15 degrees /25 degrees to 25 degrees /35 degrees ranged from nil to 53% in the different cultivars. A significant negative correlation was found between EUE at 35 degrees and the rate of plant growth rate reduction from low to high temperature, indicating an association across cultivars between heat tolerance in carbon utilization during seed germination and heat tolerance in growth of the autotrophic plant.

The effect of heat stress on the efficiency of using the endosperm of wheat parental genotypes and segregant populations was evaluated and the stress index was calculated by Cargnin *et al.*, (2006). They found that high temperature at seed germination reduced the dry matter of the plantlet and efficiency in using the endosperm. The level of heat tolerance varied among the parents and populations. Sikder *et al.* (2004) found a profound effect of temperature on seed reserve mobilization efficiency (SRME) in ten wheat varieties. With increasing temperature the SRME was decreased. At high temperature stress (32°C) varieties Balaka, Barkat and Akbar had the highest SRME (0.86 to 0.93 g/g) as compared to other varieties (0.44 to 0.73 g/g). Hasan *et al.* (2004) found a relationship between seed reserve mobilization efficiency and heat tolerance of wheat. At 25°C temperature the seed reserve mobilization efficiency was significantly higher than that at 15 and 35°C both in tolerant and sensitive wheat genotypes. But the heat sensitive genotypes had higher efficiency (2.58 to 3.48 g/g) than that of the heat tolerant genotypes (1.99 to 2.09 g/g) at 25°C. Based on reduction of efficiency both at 15 and 35°C from that of 25°C,

Sonora, CB-24 and CB-34 (HS genotypes) were found more affected than Aghrani, Kanchan and CB-30 (HT genotypes).

2.7. Proline accumulation in relation to heat tolerance

Plants incorporate 22 amino acids into their proteins. However, many plants also contain unusual amino acids, called non protein amino acids that are not incorporated into protein but are present instead in free form and act as protective substances. They are the organic compounds that do not interfere with enzyme functions and are known as compatible solutes. Proline is a non protein amino acid and compatible solute commonly accumulates in many plants exposed to various stress conditions such as water stress (Barnett and Naylor, 1966; Blum and Ebercon, 1976), salinity (Stewart and Lee, 1974; Treichel, 1975), air pollution (Godzic and Linskens, 1974) and unfavourable temperature (Chu *et al.*, 1974; Chu *et al.*, 1978). Chaitanya *et al.* (2001) found that proline accumulation was 1.5 fold higher in high temperature stressed mulberry leaves. Stewart and Lee (1974) postulated that under some circumstances, and in some plants, proline might be accumulated until it constitutes more than 10% of the dry weight of the stressed tissue, a concentration rarely exceeded by any single cellular components.

Under stresss condition, proline is synthesized from glutamate due to loss of feedback regulation in the proline biosynthetic pathway (Boggess and Stewart, 1980). This biosynthesis might be an adaptive mechanism to reduce the accumulation of NADPH, which increased as result of the decrease in photosynthetic CO_2 reduction rate of the plant (Berry and Bjorkman, 1980). Proline has also been found to serve as a substrate for respiration (Britikov *et al.*, 1965) and as a source of nitrogen and other metabolites (Stewart and Boggess, 1978). The relationship between proline accumulation and

environmental stress suggests that proline could have a protective function. Recently, Paleg *et al.*, (1981) demonstrated that a number of solutes, including proline, protected enzymes, isolated from various tissues, from inactivation by heat. Rapid catabolism of proline upon relief of stress may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress and repair of stress induced damage (Hare and Cress, 1997). The extensive accumulation of active oxygen species and their contribution to cell damage induced by environmental stress is well known. In order to deal this effect, plants have evolved a number of protective scavenging or antioxidant defense mechanisms. Apart from the enzymatic defense system (Bowler *et al.*, 1992), the accumulation of free proline may also contribute to the scavenging of these active oxygen species by enhancing photochemical electron transport activities (Alia *et al.*, 1991).

Under heat stress greater increase in proline accumulation in 6 cotton cultivars was obtained by Ronde *et al.* (2001). Takeda *et al.* (1999) observed accumulation of free proline in the leaves of Brassica vegetables in response to high temperature (35/30°C) as a possible mechanism to suppress heat stress. Kuo *et al.* (1986) reported that proline content in anthers increased with advancing development of floral buds to a maximum at anthesis. They found that the pistil had less proline compared to anthers and could not accumulate with advancement of floral bud development in most of the cultivars under study. High temperature caused higher proline content in leaves. Normally the proline content of leaves was lower than that of anthers or pistils. They subsequently suggested that low proline accumulation in the leaves might be the result of high accumulation in the floral buds. Again, anthers need high proline to confer heat resistance to pollen germinating at high temperatures. Wojciechowska *et al.* (2000) determined free proline in five strains of white cabbage and found that free

proline accumulated in cabbage heads stored both at low and high temperature. The considerable increase in proline level during short-term storage at room temperature was due to temperature rather than to water stress. Long term storage in a cold chamber induced proline accumulation. Proline accumulation in high temperature has also been reported in barley and radish leaves (Chu *et al.*, 1974), , in the leaves of Brassica vegetables (Takeda, 1999), in cabbage and Chinese cabbage (Hossain *et al.*, 1995), in apple (Park *et al.*, 2001) and in flag leaves of wheat (Hasan *et al.*, 2007).

Genotypic variations in proline accumulation have been observed in many studies and attempts were made to correlate proline accumulation with tolerance of plants to stress. Under supra-optimal temperature genotypic difference in proline accumulation pattern has also been reported in six cotton cultivars (Ronde *et al.*, 2001), in different cabbage and Chinese cabbage varieties (Hossain *et al.*, 1995) and in five cucumber cultivars (Zhang *et al.*, 1998). The proline concentration in citrus leaves differed between species and cultivars in response to temperature. As the maximum air temperature increased during summer months (42-44°C) and the minimum air temperature decreased during winter months (around 5-8°C) the leaf proline content of orange cultivars gradually increased in both seasons to 12-13.6 and 13.8-14.8 mg/g (dry wt basis) in July and January, respectively. They also found that proline concentrations were significantly higher in young leaves than in old leaves in response to air temperature stress (Sabbah *et al.*, 1996). Hasan *et al.* (2007) reported genotypic difference in increase in flag leaf of heat tolerant and heat sensitive wheat genotypes due to post anthesis heat stress condition.

2.8. Stem reserve utilization in wheat in relation to heat tolerance

Wheat grain filling depends on two major sources of carbon: current photosynthesis in leaves and ears, and the mobilization of stored reserve from the stem in to the growing grain. Stored assimilates is an important source for grain filling in wheat. Up to 0.78g of grain may be produced from 1g of assimilates stored in wheat stover (Kiniry, 1993). When the current photosynthetic source is inhibited, grain filling becomes more dependent on mobilized stem reserves (Bindinger *et al.*, 1977; Blum, 1988). Haque (2002) found photosynthate stem reserve translocation (PSR) from stem to kernel varied from 25.05 to 30.72% in heat tolerant wheat genotypes and 19.95 to 25.12 in heat sensitive genotypes under normal growing condition but under late growing condition, PSR was 23.27 to 34.02% in heat tolerant genotypes and 36.12 to 41.05% in heat sensitive genotypes.

Blum *et al.* (1994) evaluated two spring wheat cultivars (V5 and V2183) for grain growth, stem dry matter and stem total non-structural carbohydrates (TNC) loss under both non-stressed and heat stressed conditions. V5 sustained a smaller reduction in grain DW under heat stress, than V2183. Irrespective of temperature, V5 had a higher stem DM and TNC content at the onset of grain filling, greater depletion of stem dry matter and TNC during grain filling and longer duration of grain filling than V2183. During grain filling V5 generally exported about two or three times more dry matter from the stem than V2183, under both non-stressed and stressed conditions. It is concluded that the superior capacity of V5 for grain filling from mobilized stem reserves is a constitutive trait which supports grain filling under heat stress.

Shukla *et al.* (1997) indicated that greater stem reserve mobilization took place in tolerant genotypes to support grain filling during critical stages of dry matter accumulation in the grain. Thus, such mobilization is an associated desirable trait in

providing tolerance to the post anthesis heat stress in wheat genotypes. Nine wheat genotypes were evaluated by Tahir and Nakata (2005) for their ability to accumulate and remobilize total non- structural carbohydrates (TNC) stored in their stem to support grain filling under heat stress. They reported that heat stress significantly increased TNC remobilization efficiency and significant variation were observed among genotypes. TNC remobilization efficiency under heat stress positively correlated with mainstem grain yield and grain weight.

2.9. Photosynthetic activity and heat tolerance of wheat

Photosynthesis is one of the most sensitive processes to heat stress in wheat (Al-Khatib and Paulsen, 1984; Harding *et al.*, 1990; Sayed *et al.*, 1989a). Whole plant photosynthetic rates decline rapidly when plants were stressed during vegetative and reproductive phases (Bird *et al.*, 1977; Grover *et al.*, 1986; Todd, 1982; Gupta *et al.*, 2006). Thylakoid membranes activities in wheat are more affected by high temperature than are stomatal conductance and stromal enzyme components (Al-Khatib and Paulsen, 1989; Kobza and Edwards, 1987; Monson *et al.*, 1982; Sayed *et al.*, 1989b). The photosystem II (PSII) reaction center is especially labile and is damaged mostly near the water oxidizing complex (Al-Khatib and Paulsen, 1989; Harding *et al.*, 1990). The relationship between photosynthesis and genotypic variability in wheat productivity under high temperatures is unclear. Leaf photosynthetic rates differed over five fold in cultivars of tetraploid and hexaploid wheat at high temperature, but yields were not reported (Blum, 1986).

Senescence of wheat are delayed until physiological maturity when plants are grown below 20°C, but degredative processes accelerate and photosynthetic activities decline as the temperature rises. Even mildly elevated temperatures during reproductive growth are injurious in this respect and seriously reduce economic yield (Al-Khatib

and Paulsen, 1984). Heat tolerant cultivars retain higher amount of chlorophyll for longer and higher kernel dry weight in than heat sensitive cultivars under high temperature stress (Fokar *et al.*, 1998b; Haque, 2002; Gupta *et al.*, 2006). Heat stress decreased mean photosynthetic rates by 32 and 11%, chlorophyll variable fluorescence by 42 and 11% and mean total biomass by 32 and 15% in seedling and maturing plants, respectively when 10 genotypes from major world wheat producing regions were exposed to moderate and high temperature for 2 weeks at seedlings or from anthesis to maturity (Al-Khatib and Paulsen, 1990).). High temperature (41°C) causes a decrease in the potential photosynthetic rate in both heat resistant and susceptible varieties although the decrease was smaller and recovery ability was higher in the resistant variety (Volkova and Koshkin, 1984).

The sharp decrease in SPAD value indicates the onset of leaf senescence. Rajcan *et al.* (1999) reported that leaf senescence of maize occurred at a SPAD reading between 25 and 30. Mohi-Ud-Din *et al.* (2007) found that the onset of flag leaf senescence of two wheat cultivars was indicated by the SPAD value reaching below 30 and by the highest level of soluble carbohydrate accumulation in the flag leaf. The onset of flag leaf senescence commenced earlier in heat sensitive Sonora than in heat tolerant Kanchan under heat stressed environment (Mohi-Ud-Din *et al.*, 2007). Excess non-structural carbodyhrate accumulation in flag leaf inhibits leaf photosynthesis via feedback effects (King *et al,* 1967 and Azcon-Bieto, 1983) and also accelerates leaf senescence in wheat (Lazan *et al.*, 1983).

The activity of peroxide (POD) increased significantly in wheat flag leaf due to heat stress which probably damaged the leaves and accelerated their senescence. Two enzymes: superoxide dismutase (SOD) and catalase (CAT) cooperated with each other generally retard the senescence of the flag leaf. In heat tolerant wheat

genotypes, the activity of SOD and CAT was increased significantly to retard senescence of flag leaf under stressed condition (Jiang *et al.*, 2007). Among five wheat genotypes, the heat tolerant HD 2815 and HDR-77 showed the highest activity of various antioxidant enzymes (superoxide dismutase, ascorbate peroxidase, catalase) under late and very late sowing also showed minimum reduction in chlorophyll content and lower membrane injury index, indicating the amelioration of high temperature stress induced oxidative stress by antioxidant enzymes. Various antioxidant enzymes showed positive correlation with chlorophyll content and negative with membrane injury index at most of the stages in five wheat genotypes (Almeselmani *et al.*, 2006).

2.10. Kernel growth physiology and heat tolerance

Grain yield of wheat per unit ground area depends on number of kernels and kernel weight. Final kernel weight depends, in turn, on rate and duration of grain filling (Gallagher *et al.*, 1976). Under high temperature, the wheat crop completes its cycle much faster than under normal temperature conditions (Fischer, 1985). Consequently, there are fewer days to accumulate assimilates during the life cycle and production of biomass is reduced (Fischer and Maurer, 1978).

Generally reduced kernel weight in heat stressed wheat results from shorter duration rather than reduced rate of grain growth (Sofield *et al.*, 1977; Warringhton *et al.*, 1977; Wiegand and Cuellar, 1981; Zahedi and Jenner, 2003; Sial *et al.*, 2005). Tashiro & Wardlaw (1989) reported lower grain weight in heat stress due to reduction in the grain filling period and grain filling rate or the combine effect of both. They also found a reduction in the grain growth duration and dry matter accumulation rate with increasing temperature above a mean of 26.7°C. Each degree Celsius in temperature

during grain filling results in about three day reduction in the duration of grain filling regardless of cultivars (Asana and Williams, 1965; Begga and Rawson, 1977). Genotypic variation also exists in response to high temperature for the rate and duration of grain filling (Zahedi and Jenner, 2003; Sikder *et al.*, 1999; Jhala and Jadon, 1989; Viswanathan and Chopra, 2001). Different wheat genotypes maintain a higher growth rate (more than 1 mg/day/kernel) for longer time under normal condition but post anthesis heat stress reduces this duration. The HT genotypes possess rapid kernel growth duration for longer time than the HT genotype (Hasan and Ahmed, 2005).

Data on dry matter and starch accumulation in wheat grain are fitted to a sigmoid curve that reveals a close correspondence between two parameters. Wheat grain contains higher amount of soluble sugar at early reproductive stage but declines with advances in growth up to almost middle of grain growth, even becomes high at later stage (Chanda *et al.*, 1999; Hasan and Ahmed, 2005).

Although the early stages of grain development are sensitive to high temperature (Jones *et al.*, 1981; Tashiro and Wardlaw, 1990a), there are also direct effects on starch deposition (Bhullar and Jenner, 1986) which are apparently not due to a limitation in the supply of assimilate to the grain (Sofield *et al.*, 1977; Wardlaw *et al.*, 1980; Hasan and Ahmed, 2005). Starch synthesis is highly sensitive to high temperature stress due to the susceptibility of starch synthase in the developing kernels of wheat (Denyer *et al.*, 1994; Jenner, 1994). MacLeod and Duffus (1988) indicated that elevated temperature significantly reduces the activity of the sucrose cleavage enzyme UDP sucrose synthase found in the endosperm during grain development and these effects may be initiated by a relatively short period of thermal stress applied close to anthesis.

During the early period of grain filling, the activities of key enzymes such as sucrose synthase (SS) and granule-bound starch synthase (GBSS) are enhanced, but the activities of sucrose synthase (SS), granule-bound starch synthase (GBSS) and soluble starch synthase (SSS) during the late period of grain filling are reduced due to high temperature (Zhoa *et al.*, 2006). ADP-glucose pyrophophorylase controls the synthesis but the effects of reduced ADP-glucose pyrophophorylase on starch synthesis appeared to be exerted primarily through a reduction in grain filling duration rather than on grain filling rate (Singletary *et al.*, 1994). Keeling *et al* (1994) reported soluble starch synthase (SSS) as the major site of control of flux through the pathway of starch synthesis in developing wheat grain and temperatures above 25°C adversely affect flux and limit yield.

2.11. Yield and yield contributing characters and heat tolerance

Heat stress adversely affects the plant growth and thus limits wheat productivity in many region of the world, particularly countries in the tropical belt between 23°N and 23°S latitude (Fischer and Byerlee, 1991). High temperatures accelerate organ production without any increase in net photosynthesis (Begga and Rawson, 1977) resulting in smaller organs. Most of the morphophysiological characters are altered when planting was delayed by one month from the normal seeding date (Bhatta *et al.* 1994).

Productivity of wheat reduces at high temperature (Bhullar and Jenner, 1983; Rawson, 1986; Shpiler and Blum, 1986; Wardlaw *et al.*, 1980; Hasan and Ahmed, 2005; Poonam *et al.*, 2006). Evans *et al.* (1975) and Mudholkar (1981) reported that higher temperature at grain filing stage was one of the important reasons for lower grain yield in wheat crop. Wheat genotypes incur differential injury from high temperatures during vegetative growth (Shpiler and Blum, 1986), reproductive growth (Bhullar and Jenner, 1983;

Wardlaw *et al.*, 1980), or both periods (Rawson, 1986). Many wheat genotypes can be considered high temperature tolerant (Rawson, 1986).

Number of grains per spike is determined during GS_2 phase (double ridge to anthesis). This is the most sensitive stage and its duration is drastically reduced due to heat stress. There is a corresponding decrease in spikelet/ floret number per spike and grain number per unit area. Assuming that grain number is solely controlled by prevailing temperature, Abrol *et al.* (1991) observed a linear regression of grain number on the mean temperature from sowing to anthesis. The regression shows a reduction of 5.5% in grain number for every 1°C increase in temperature. Acevedo *et al.* (1991) observed detrimental effects of high temperature on grain number and the duration of spike development during GS_2 stage. Shpiler and Blum (1986) observed that the cultivars that sustained the highest yield in hot environments were able to maintain the longest duration of GS_2 and had higher number of grain per spike. High temperature (up to 30/25 °C day/night) after anthesis reduced grain yield of wheat by reducing weight per grain rather than grain number (Wardlaw *et al.*, 1989).

Al-Khatib and Paulesn (1990) found decreased kernel weight significantly in different wheat genotypes by a mean of 20% whereas the reduction range was about 10% to 30%. Grain yield per ear declined from 0.75 to 0.58 g or 23% from 22/17 to 32/27°C temperature. Yields were constant for 3 genotypes and decreased >40% for three genotypes. Harvest index of different genotypes was affected little by temperature, but individual genotypes responded differently. Correlation studies indicates that flag leaf sheath length, extrusion length, plant height, spike length, kernel per spike and biological yield are important determinants of grain yield in wheat under Los Banos conditions (Fischer and Maurer 1976).

Fokar *et al.* (1998b) observed significant variation among five wheat cultivars in the reduction in grain weight per ear, kennel number and single kennel weight under heat stress. Differences in grain weigh per ear among cultivars were ascribed to the variation in the reduction in both kennel number and kernel weight under heat stress.

Islam *et al.* (1993) evaluate the performance of the existing (Sonalika) and released wheat varieties (Ananda, Kanchan, Barkat, Akbar, Aghrani) seeded from 1 November to 15 January at 15 days interval. Grain yield, spike/m^2, grain/spike and 1000-grain weight were significantly affected by sowing date and variety. The highest grain yield was obtained with variety Kanchan when sown on 15 November which was identical to Akbar and Barkat. Ahgrani performed better than all other varieties when sown in December and January. Sonalika variety also showed lower yield than the other varieties when seeding was done in December and January. Different yield component of these 6 varieties at maturity reacted differently to late seeded conditions. Delay sowing caused significant reduction in grain weight due to higher temperature at grain filing stage.

Chinoy (1947) compared grain weight per plant and 1000 grains weight of wheat varieties grouped into eight classes according to flowering time, which ranged from 90-100 to 160-170 days. With delay in flowering, grain developed at increasingly higher temperatures and lower humidity, with the consequence that both 1000 grain weight and grain yield per plant diminished progressively. Pal and Butany (1947) also concluded that 1000 grain weight was reduced for late sowing because of the high temperatures prevalent at the time of grain ripening. Chinoy and Sharma (1957, 1958) found that external conditions such as temperature were mainly responsible for under developed or empty grain. In a series of pot experiments, Asana and Saini (1962) compared the 1000-grain weight for two varieties of wheat, Pb.C. 281 and N.P. 720. They observed that 13.9 and 17.8% losses in grain weight for Pb.C. 281 and N.P. 720 respectively for a 5°C rise

in the mean temperature and assuming that yield attribute is determined solely by temperature.

Hasan and Ahmed (2005) found that grain yield reduced by about 2.6 to 5.8% for each 1°C rise in average mean air temperature from normal growing condition during anthesis to maturity in HT genotypes but it was about 7.2% in heat sensitive Sonora. Based on grain yield, the heat tolerant genotypes showed lower susceptibility index (0.346 to 0.962) than the heat sensitive genotype (1.721). Heat stress reduced the grain yield but low heat susceptibility indexes for grain yield were found in heat tolerant genotypes (Rasal *et al.*, 2006). Late sowing of wheat reduced grain yield by 30 to 40% compared to November sowing in India (Poonam *et al.*, 2006). With delay planting, the development of plant organs and transfer from source and sink were remarkably affected, which was reflected by overall shortening of plant height and ultimately in the reduction of yield and yield component (Sial *et al.*, 2005). Biomass reduces by 40%, grain number on main tiller by 41% and individual grain weight by 45% when wheat plants are subjected to a heat stress at anthesis stage. The harvest is reduced from 0.53 to 0.33 (Wollenweber *et al.*, 2003).

2.12. Nitrogen management to sustain wheat yield under heat stressed environment

An on-farm experiment was conducted at Willbriggie, New South Wales, Australia to investigate the advantages of extra nitrogen topdressing in wheat production by Menzies and Whitworth (2004). A 1.0 t/ha yield increase resulted from a second nitrogen topdressing at the first node stage, which also gave an economic response of $A 88/ha. There was no significant different in protein content between single and double topdressings. Topdressing a third time gave a 0.45 t/ha yield response and increased protein content but no economic benefit was gained. Malhi and Kutcher

(2004) also found increase seed yield, protein content and N uptake with increased N rate in North Central Saskatchewan. The seed quality factors such as thousand kernel weight, bulk density and % plump seeds were declined as N rate increased in stressed condition.

Badaruddin *et al.* (1999) reported that 50% extra N increased ear/m^2 by 6%, total biomass by 29% but plant height did not influenced by extra nitrogen. They also reported that extra 50% N increased 8% wheat yield at normal sowing and 17% under hotter late-sown condition over control in Mexico. Comparison of heat tolerant (Glennson 81) and heat sensitive (Pavon 76) genotypes showed that the heat tolerant genotype was generally more responsive to additional inputs.

CuiPing *et al.* (2007) showed that increasing ratio of top dressing to basal nitrogen was beneficial to grain weight under both normal air temperature and heat stress, and it lessened the adverse effects on 1000 grain weight and grain yield under stress condition.

2.13. Effect of heat stress during seed development and maturation on seed quality

Seeds obtained from different seasons or different geographical areas often vary in germination capacity and viability. Much of this variation has been attributed to differences in environmental conditions prevailing during the formation, development and maturation of the seed while still on the mother plant (Peacock and Hawkins, 1970; Datta *et al.*, 1972). Several workers have reported on the influence of different types of environmental stress on seed quality e,g., drought stress (Dornbos *et al*, 1989), high temperature (Siddique and Goodwin, 1980; Keigley and Mullen, 1986), freezing (Judd *et al.*, 1982) etc.

High temperature is one of the most important environmental factors affecting not only plant growth and development but also the quality characteristics of the progeny. High temperature accelerates the initial growth rate but shorten the grain growth period. As a consequence, the final seed size is much reduced at high temperature. The percentage of smaller, etched, discolours and wrinkled seed was greater in soybean when it was grown at high temperature during seed growth and maturation (Keigley and Mullen, 1986). Smaller seed from high temperature was also reported for white seeded bean by Moss and Mullett (1982). Soybean seed developed under high temperature stress (Dornbos and Mullen, 1991) had greater percentage of hard seed. Many seed produced by soybean plants exposed to excessive high temperature during seed filling are shriveled or abnormal. Significant levels of shriveled and abnormal seed would reduce the quality of a seed lot (Spears *et al.*, 1997). Khan and Laude (1969) reported that the physical properties of seed including seed size, seed dimension, seed coat and appearance of the barley seed were affected by high parent growth temperature. High temperature during seed development reduced grain density in maize (Lu *et al.*, 1993).

In addition to the effects of high temperature on physical properties of seed, physiological quality is also strongly influenced. Previous studies have demonstrated that high temperature during seed development and maturation decrease seed dormancy in several species, such as in rye (Lalluka, 1976), lettuce (Gray *et al*, 1988). Several workers have also shown that heat stress may decrease seed germination and/or seedling vigour. High temperature during soybean seed fill reduces germination and vigour of mature seed (Keigley and Mullen, 1986; Egli *et al.*, 2005). Fussel and Pearson (1980) found that the temperature at which pearl millet grain development did not affect seed viability, but grains developed at low temperature

produced more vigorous seedling s than grain development at high temperatures. Steiner and Opoku-Boateng (1991) demonstrated that high air temperature shortly before and after anthesis reduced vigor of mature lettuce seed. Siddique and Goodwin (1980) found that high temperature (33°C) during seed growth reduced seed quality of bean. Abdul Baki and Anderson (1973) has reported that any decrease in the metabolic activity of the excised embryo of soybean will be directly associated with a loss in seed vigor. Madden (1992) showed that high temperature during seed development altered respiration rates in imbibed maize axes. Grain development at elevated temperature can affect membrane integrity and can cause increase in membrane leakage of both electrolytes and macro-molecules during germination which subsequently impair germination and seedling vigor (Girelberg *et al.*, 1984).

High temperature during seed development also affects wheat seed quality. High temperature following anthesis adversely affects grain development in wheat (Tashiro and Wardlaw, 1990a; Hasan and Ahmed, 2005). High temperature accelerates the initial grain growth rate but shorten the grain growth period (Sofield, 1977; Hasan and Ahmed, 2005). As a result, smaller and shrunken grain is produced at high temperature (Tashiro and Wardlaw, 1990b). Seed dormancy in wheat also reduced due to high temperature during seed development (Olsson and Mattsson, 1975; Reddy, 1985). Grass and Burris (1995a) reported genotypic difference in reduction of wheat seed germination due to heat stress during seed development and maturation. They also reported decline in seed vigor in wheat due to heat stress of mother plant which was reflected in reduced shoot and root dry weight, increase shoot to root ratio and higher seed conductivity. Exposing seeds to high temperature during development and maturity also resulted in low embryo oxygen uptake. Sechnyak *et al.* (1985) showed significant influence of average daily air temperature on developing seed

quality, 1000 grain weight, rate and evenness of germination, strength of initial growth of wheat seed. Mature seeds showed nonsignificant differences in laboratory germination. Seeds developed under high temperature had a shorter and less deep dormancy. Protein content per biological unit and enzyme activity was changed by temperature increasing. Temperature influences winter wheat seed quality by regulation of the plant's photosynthetic process and source-sink systems of the organ. Similar results for barley have been published by Faris *et al.* (1969). Nucleotide levels and respiratory activity of mitochondria isolated from imbibing embryos were determined by Grass and Burris (1995b). They found that embryos from low temperature treatments showed rapid accumulation of ATP, higher energy levels and rates of oxygen uptake than embryos from high temperature treatments. Parallel to these metabolic changes during early seed germination, results from electron microscopy revealed visible differences in mitochondrial structure. Mitochondria from low temperature regime were well developed with visible membrane and cristae; those from the high temperature regime were degenerating.

CHAPTER III

Experiment 1. EVALUATION OF SEED RESERVE UTILIZATION EFFICIENCY DURING GERMINATION IN RELATION TO HEAT TOLERANCE OF WHEAT GENOTYPES

INTRODUCTION

Genotypic variation in heat tolerance of wheat in terms of yield component development and final biomass and yield was traced to the growth vigour under heat stress of the juvenile plant (Rawson, 1986; Shpiler and Blum, 1986). The heat tolerance in adult plant may be associated with heat tolerance at the seedling stage. Alkhatib and Paulsen (1990) found a high correlation across 10 wheat cultivars in photosynthetic reductions due to heat stress between the seedling stage (4 weeks) and the flowering stage. Saadalla *et al.* (1990a) suggested that heat tolerance in wheat coleoptiles was predictive of yield performance of different wheat cultivars under heat stress condition in the field. Such an association is important, as it would seem to indicate genetic control over thermotolerance that is independent of plant ontogeny.

A major perturbation to plant productivity under heat stress is ascribed to 'carbon starvation' (Levitt, 1982). Heat stress is assumed to impair the plant carbon balance by reducing photosynthetic carbon assimilation and by accelerating carbon loss to respiration. During seed germination the developing wheat seedling is totally dependent on the utilization of the carbon stored in the seed endosperm. Seed reserve utilization for seedling growth in the dark proceeds at an efficiency, which may be calculated as the ratio of the gain in seedling biomass to the loss in seed reserve. Seed reserve utilization for seedling growth in the dark may therefore be considered as a simple model for studying tolerance to heat stress in terms of the carbon balance. In a preliminary study, Hasan *et al.* (2004) and Cargnin *et al.* (2006) found that heat

tolerant wheat genotypes exhibited lesser loss of seed dry matter than sensitive genotypes during germination. The present study was therefore, conducted to evaluate the hypothesis that heat tolerance in terms of seed reserve utilization efficiency during germination may be associated with the heat tolerance in the autotrophic plant after germination.

MATERIALS AND METHODS

Location and duration

The experiment was conducted at Crop Botany Laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during the period from July to November 2006.

Wheat genotypes

Twenty wheat genotypes (Table 3.1) including most of the popular varieties, some advanced lines and some lines from abroad collected from Wheat Research Centre (WRC) of BARI, Dinajpur were used for the present study.

Table 3.1. List of wheat genotypes used for the present study

SL. No.	Genotypes	Remarks
1.	Shatabdi	Variety
2.	Kanchan	Variety
3.	Prodip	Variety
4.	Bijoy	Variety
5.	Fang 60	Variety
6.	BAW 1064	Advanced line
7.	Sufi	Variety
8.	Gourab	Variety
9.	BAW 1059	Advanced line
10.	Pavon 76	Variety
11.	Sonora	Variety
12.	BL 1883	Line from Nepal
13.	BL 1022	Line from Nepal
14.	IVT 6	Line from India
15.	IVT 7	Line from India
16.	IVT 8	Line from India
17.	Kanlyan Sona	Variety
18.	IVT 9	Line from India
19.	IVT 10	Line from India
20.	BAW 917	Advanced line

Membrane thermal stability test

Procedure used for measuring Membrane injury (%) to high temperature was the same as describe by Ibrahim and Quick (2001) with some modification. A suitable floating stage was prepared by styrofoam with twenty holes using net at the bottom. Three different stages (used as three replications) were set on water in a water bath (J. P. SELECTA, Ctra-Nil-Km- 585.1, Abrera, SPAIN) for establishment of the seedlings. The seeds of twenty wheat genotypes were germinated in petridishes using distilled water at room temperature for two days. Then fifteen pre-germinated seeds of each genotype were transferred and placed at the holes of floating styrofoam stage. The seedlings were grown at room temperature and the water in water bath was aerated continuously by air pump (AIR- PUMP, YAMANO, AP-3500). Acclimation was started when the first leaf attained about 8 to 10 cm in length (8 days after germination). The water bath was maintained at 39 °C for 48 hours and covered with transparent plastic to ensure adequate light. Following acclimation, 7 cm long leaf segments were excised from 10 seedlings per genotype to form the experimental unit. Leaf segments were rinsed twice in deionized water and placed in 25 × 200 mm test tubes with 20 ml deionized water. Severe heat stress was applied by submerging tubes to a depth equal to the height of water in the test tubes in a water bath at 49 °C for 30 minutes. After the treatment period, the test tubes were held over night at room temperature. Conductance was measured with an electrical conductivity meter (Model HI 8083, HANNA instruments, Portugal) after standardizing it with a 0.005 KCl solution. The test tubes were then autoclaved for 10 min at 121 °C and 0.10 MPa and the conductance was measured again as an indication of the maximum potential leakage from a given sample. Relative membrane injury (%) was calculated using the following formula-

$$RI\ (\%) = (T_1/T_2) \times 100$$

where T_1 and T_2 are the conductivity readings before and after autoclaving, respectively. The data were analyzed in completely randomized design and the means were separated by DMRT at 5% level.

Determination of seed reserve utilization efficiency

Before placement of seed for germination, the seeds of a genotype were thoroughly mixed and moisture percentage was determined gravimetrically using a portion of the seeds, the remaining seeds were used for the experiment. Individual weight of 10 seeds for each genotype were taken and were placed sequentially according to the marking on filter paper soaked with water in sterilized petridishes. Then the petridishes were kept in seed germinator (ATTEMPTER, Advantec, Japan) at 25 and 35^0C. For each temperature three batches of petridishes each containing 10 seeds was used. Water was added to the petridishes when necessary. At 5th day after placement for germination, five seedlings from each petridish were sampled. Then shoot, root and remaining seeds were dried separately at 70°C for 72 h and weight were recorded.

Seed reserve utilization efficiency in the present study is defined as the amount of shoot and root dry matter (g) produced from 1 unit (g) of dry seed weight that was loss as respiration. Thus higher the value of seed reserve utilization efficiency (SRUE), the higher is the efficiency of seed as more seed reserves would be used for producing roots and shoots.

Amount of seed material loss as respiration (SMR) was calculated as -

$$SMR = SDW - (SHW + RTW + RSW)$$

Where,

SDW = Seed dry weight before germination

SHW = Shoot dry weight

RTW = Root dry weight

RSW = Remaining seed dry weight

Seed reserve utilization efficiency (SRUE) was calculated as Hasan *et al.* (2004) by the following formula-

$$SRUE = \frac{SHW + RTW}{SMR}$$

Relative performance

The relative performance was calculated as Asana and Williams (1965) by the following formula-

$$\text{Relative performance} = \frac{\text{Variable measured under stress condition}}{\text{Variable measured under normal condition}}$$

Statistical analysis

The data were analyzed using two factor completely randomized design and treatment means were compared by DMRT. Correlation analysis was also done and level of significance was tested with t-test.

RESULTS AND DISCUSSION

Membrane injury to high temperature of 10 day old seedlings of twenty wheat genotypes was measured as describe by Ibrahim and Quick (2001) in the present experiment. The seed reserve utilization efficiency during germination of those genotypes was also estimated to compare the heat tolerance of wheat genotypes in terms of SRUE with membrane thermostability. The results of the present experiment in the form of tables and figures along with necessary discussion have been presented in this part.

Membrane injury to high temperature

Membrane injury (%) of 10 days old seedlings varied significantly among 20 wheat genotypes, with a range of 35.2 to 67.9% and an average of 46.90% (Fig. 3.1). These MI (%) values indicate a wide difference in heat tolerance among the genotypes, as assessed by the membrane thermostability test (Ibrahim and Quick, 2001). These wheat genotypes were separated into two groups based on membrane injury (%) values. Those genotypes producing less than 50 MI(%) were classified as heat tolerant (HT) and the wheat genotypes producing equal or greater than 50% membrane injury were grouped as heat sensitive (HS). Accordingly, nine genotypes such as Shatabdi (53.4%), Prodip (51.2%), Bijoy (59.0%), BAW 1064 (60.9%), Sufi (51.5%), Gourab (55.3%), Pavon 76 (67.9), Sonora (53.6%) and Kalyansona (51.9%) were classified HS genotypes (an average of 56.08%). The other eleven wheat genotypes i.e., Kanchan (39.2%), Fang 60 (37.8%), BAW 1059 (41.4%), BL 1883 (44.2%), BL 1022 (37.4%), IVT 6 (35.4%), IVT 7 (38.6%), IVT 8 (41.9%), IVT 9 (35.2%), IVT 10 (40.1%) and BAW 917 (42.0%) were grouped as heat tolerant genotypes (an average of 39.38%).

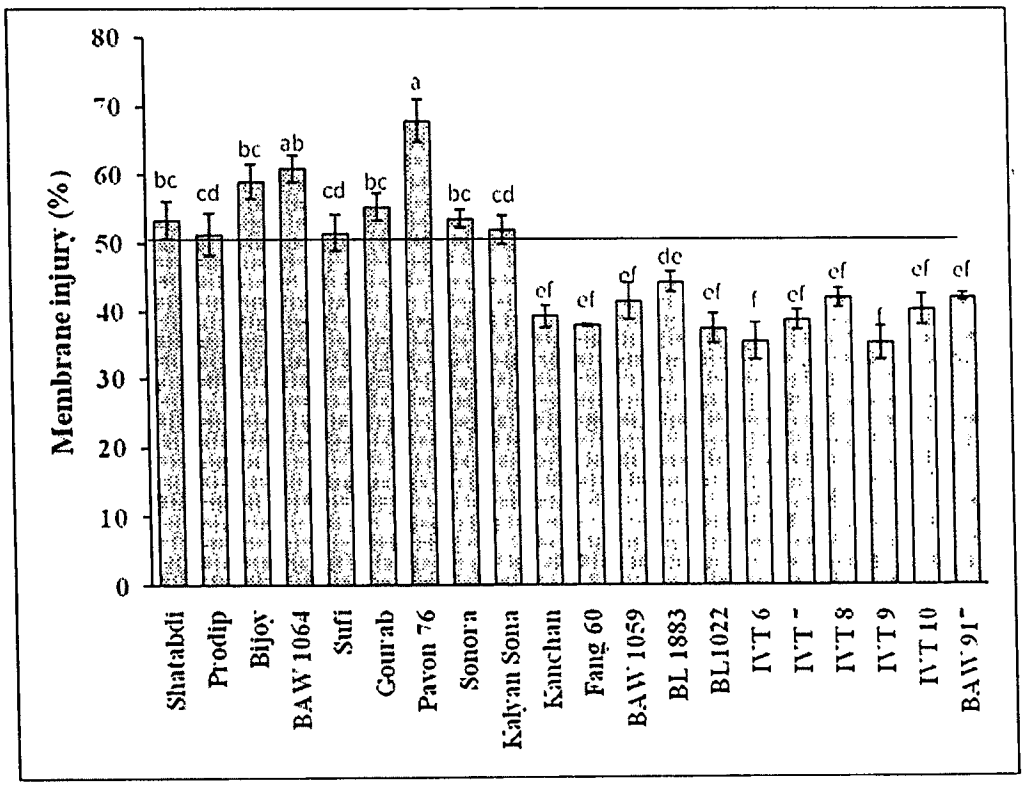


Fig. 3.1. Membrane injury (%) (mean ± SE) to high temperature of 10 days old seedling of 20 wheat genotypes.

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

Loss of membrane integrity could be a major reason of ion leakage from the cells, this phenomenon could also caused by thermally induced inhibition of membrane bound enzymes responsible for maintaining chemical gradients in the cell (Berry and Bjorkman, 1980). Under heat stress, the activity of superoxide dismutase and catalase increased more in heat tolerant wheat genotypes than the sensitive one (Zhou *et al.*, 1995; Jiang *et al.*, 2007; Almeselmani *et al.*, 2006). As high temperature membrane damage was caused by free radicals, the higher activity of superoxide dismutase and catalase may be important for higher membrane thermostability in tolerant genotypes. Genotypic differences in membrane injury (%) was also repoted in wheat by Blum and Ebercon, 1981; Shanahan *et al.*, 1990; Saadalla *et al.*, 1990a, Fokar *et al.*, 1998a; Ibrahim and Quick, 2001; Mohi-Ud-Din *et al.*, 2007; Hasan *et al.*, 2007; Haque, 2002; Sikder *et al.*, 1999. In many of these studies Kanchan was also classified as HT and Sonara and Pavon 76 were as HS genotypes.

Seed dry matter distribution during germination

The proportion of seed dry matter accumulation in shoot and root, the amount of dry matter loss as respiration during germination and the dry matter remain in seed at 5 days after placement of germination were significantly influenced by the interaction effect of temperature regimes (25°C and 35°C) and wheat genotypes (Table 3.2).

The seed dry matter distribution to shoot was significantly higher at 25°C, with a range of 15.4 to 28.5% and a mean of 21.78% compared to that at 35°C (with a range of 5.0 to 9.1% and a mean of 7.17%). But the reduction in shoot dry matter from 25°C to 35°C, was more or less same both in HS (from 20.94 to 6.88%) and HT (from 22.48 to 7.41%) wheat genotypes (Fig. 3.2 and 3.3).

Table 3.2. Seed dry matter distribution (%) of twenty wheat genotypes (5 days after placement of germination) as influenced by two temperature regimes

Genotypes	Seed dry matter distribution							
	Shoot (%)		Root (%)		Respired (%)		Remain in seed (%)	
	25°C	35°C	25°C	35°C	25°C	35°C	25°C	35°C
Shatabdi	22.0ce	6.5hl	14.2eg	4.3jm	22.8dh	14.8jl	40.9hk	74.5bc
Prodip	17.7fg	6.7hk	13.8fh	3.4kn	21.3fh	14.2jm	47.1fg	75.7bc
Bijoy	17.7fg	8.0hk	12.9gi	4.8jk	28.7ab	20.1gh	40.7hk	67.2e
BAW 1064	22.3ce	9.1h	19.9a	3.4n	23.9cf	14.1jm	33.9ln	73.4cd
Sufi	15.8g	7.3hl	11.5i	3.2kn	25.3ce	15.7jk	47.4fg	73.8c
Gourab	15.4g	5.0l	14.8df	2.3n	20.3gf	11.2mq	49.5f	81.5a
Pavon 76	27.5a	5.4kl	17.1b	3.3ln	25.8bd	16.8ij	29.6n	74.4bc
Sonora	26.8ab	6.3hl	17.1b	5.6j	23.2dg	19.6hi	32.9ln	68.6de
Kalyan sona	23.1c	7.6hl	13.2fi	3.8jn	22.2eh	15.9jk	41.4hj	72.8cd
Kanchan	22.6cd	7.1hl	16.9bc	4.1jm	29.3a	13.2kn	31.3mn	75.6bc
Fang 60	20.1df	7.2hl	15.9bd	3.9jn	26.9ac	10.8nq	37.2 il	78.1ac
BAW 1059	18.5f	5.9jl	13.7fh	2.7mn	24.0cf	9.8ko	43.8 gh	81.6a
BL 1883	19.9ef	7.5hl	12.8gi	4.0jn	17.1ij	12.8mq	50.1f	75.7bc
BL 1022	20.2df	7.5hl	15.5ce	3.6kn	22.2eh	11.4lp	42.14hi	77.5ac
IVT 6	23.5c	7.8hl	13.9eh	4.8jk	27.0ac	11.6q	35.5km	75.8bc
IVT 7	23.8c	7.6hl	16.2bd	4.8jk	22.7dh	12.4lp	37.3il	75.2bc
IVT 8	24.6bc	6.2il	12.4hi	3.4kn	27.0ac	8.6pq	35.8km	81.8a
IVT 9	22.9c	8.6hj	12.5hi	4.2jm	22.1cf	12.2nq	40.5 hk	75.1bc
IVT 10	26.6ab	7.1hl	12.4hi	4.0jn	22.9dg	9. 5pq	38.2ik	79.5ab
BAW 917	24.6bc	9.0hi	17.2b	4.61jl	21.7fh	10.4nq	36.6jl	75.9bc
Mean	21.78	7.17	14.70	3.91	23.82	13.26	39.59	75.69
CV%	10.02		9.80		9.11		4.92	

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

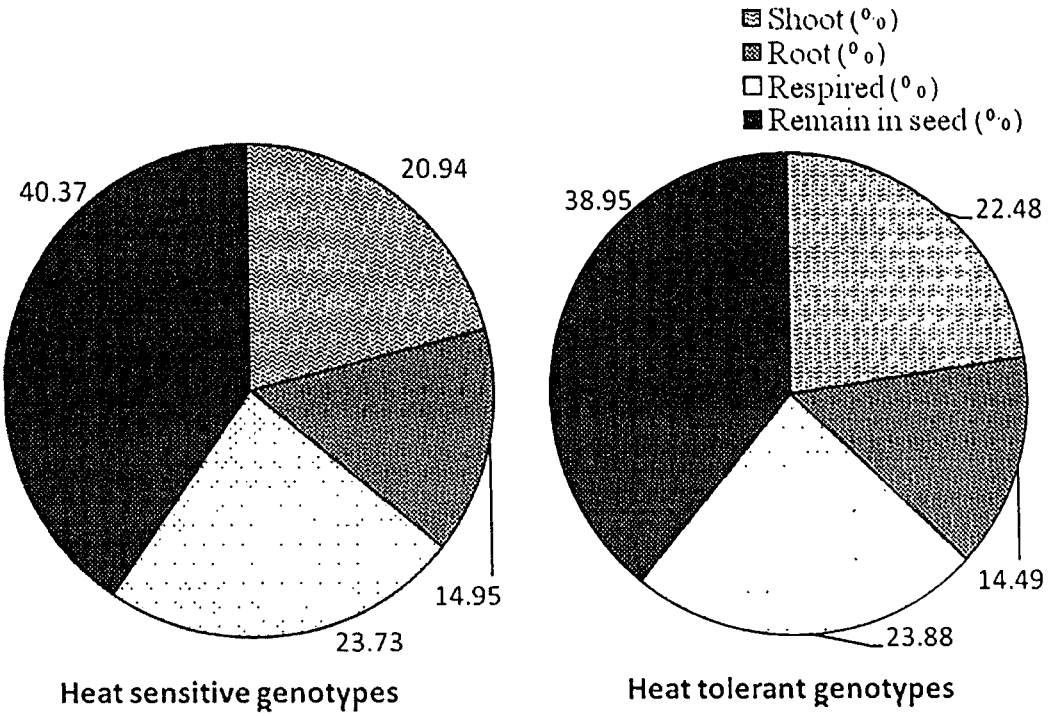


Fig. 3.2. Seed dry matter distribution (5 days after placement of germination) of heat sensitive and heat tolerant wheat genotypes at 25°C.

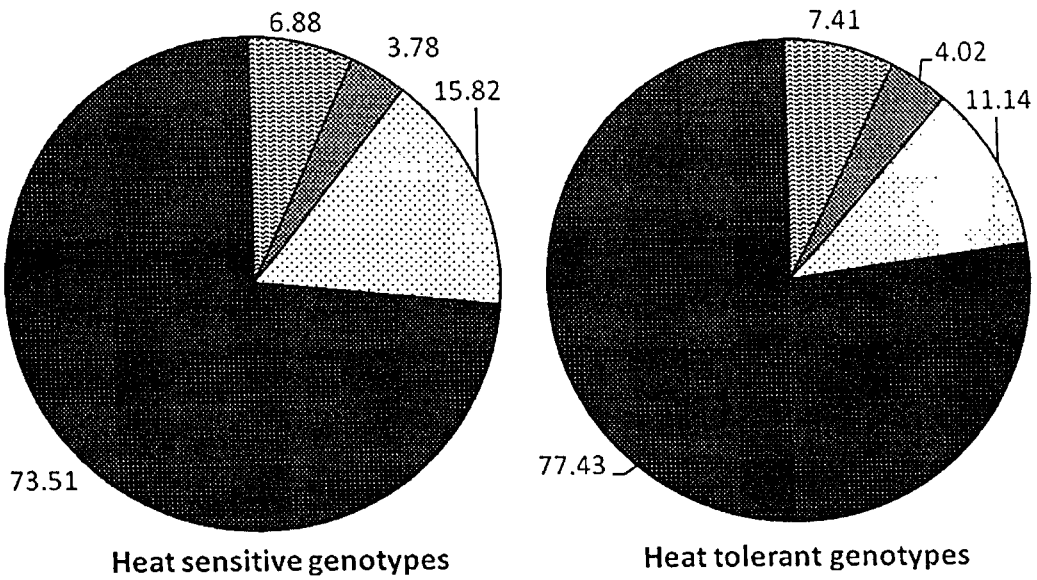


Fig. 3.3. Seed dry matter distribution (5 days after placement of germination) of heat sensitive and heat tolerant wheat genotypes at 35°C.

The proportion of seed dry matter translocation to root was significantly higher at 25°C (with a range of 11.5 to 19.9% and a mean of 14.70%) compared to that at 35°C (with a range of 2.3 to 5.6% and a mean of 3.91%). Again the reduction in root dry matter from 25°C to 35°C, was more or less same both in HS (from 14.95 to 3.78%) and HT (from 14.49 to 4.02%) wheat genotypes (Fig. 3.2 and 3.3).

The amount seed reserve loss due to respiration during germination decreased significantly from 25°C (with a range of 17.1 to 29.3% and a mean of 23.82%) to 35°C (with a range of 8.6 to 20.1% and a mean of 13.26%) in all wheat genotypes. The seed reserve loss at 25°C was more or less same in HS (23.73%) and HT (23.88%) genotypes but at 35°C, higher respiratory loss was found in HS genotypes (15.82%) compare to that in HT genotypes (11.14%) (Fig. 3.2 and 3.3).

At 35°C, the remaining seed contained significantly higher amount of seed reserve (with a range of 67.2 to 81.8% and a mean of 75.69%) compare to that at 25°C (with a range of 29.6 to 50.1% and a mean of 39.59%). The amount of reserve remain in seed at 25°C was more or less same in HS (40.37%) and HT (38.95) genotypes but at 35°C, the remaining seed contained lower amount of dry matter in HS genotypes (73.51%) compare to that in HT (77.43%) genotypes (Fig. 3.2 and 3.3).

Seed reserve utilization efficiency during germination

Seed reserve utilization efficiency (SRUE) of dark grown wheat seedlings was significantly influenced by the interaction effect of temperature regimes and wheat genotypes (Table 3.3). At 25°C temperature, all wheat genotypes showed significantly higher SRUE during germination (with a range of 1.062 to 1.931 g/g and a mean of 1.549 g/g) compare to that at 35°C (with a range of 0.507 to 1.348 g/g and a mean of

Table 3.3. Seed reserve utilization efficiency (mean ± SE) of twenty wheat genotypes (5 days after placement of germination) as influenced by temperature regimes

Genotypes	Seed reserve utilization efficiency (g/g)		
	At 25°C	At 35°C	Relative to 25°C
Shatabdi	1.596±0.05 bc	0.736 ±0.06 jl	0.46
Prodip	1.501±0.01 cd	0.715±0.01 jl	0.48
Bijoy	1.062±0.06 fi	0.629l±0.01 m	0.59
BAW 1064	1.767±0.07 ab	0.896±0.03 gj	0.51
Sufi	1.080±0.03 fh	0.673±0.02 km	0.62
Gourab	1.481±0.05 cd	0.651±0.04 lm	0.44
Pavon 76	1.751±0.14 ab	0.507±0.07 m	0.29
Sonora	1.905±0.08 a	0.595±0.03 lm	0.31
Kalyan Sona	1.636±0.01 bc	0.737±0.03 jl	0.45
Kanchan	1.343±0.05 de	0.854±0.03 ik	0.64
Fang 60	1.336±0.01 de	1.034±0.09 fi	0.77
BAW 1059	1.347±0.03 de	0.885±0.04 hj	0.66
BL 1883	1.917±0.05 a	0.903±0.05 gj	0.47
BL 1022	1.608±0.04 bc	0.978±0.02 fi	0.61
IVT 6	1.398±0.10 d	1.096±0.09 fg	0.78
IVT 7	1.768±0.01 ab	1.029±0.09 fi	0.58
IVT 8	1.370±0.04 de	1.103±0.04 fg	0.81
IVT 9	1.478±0.11 cd	1.057±0.04 fi	0.72
IVT 10	1.701±0.05 b	1.176±0.10 ef	0.69
BAW 917	1.931±0.05 a	1.348±0.16 de	0.70
Mean	1.549	0.880	-
CV(%)	8.86		-

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

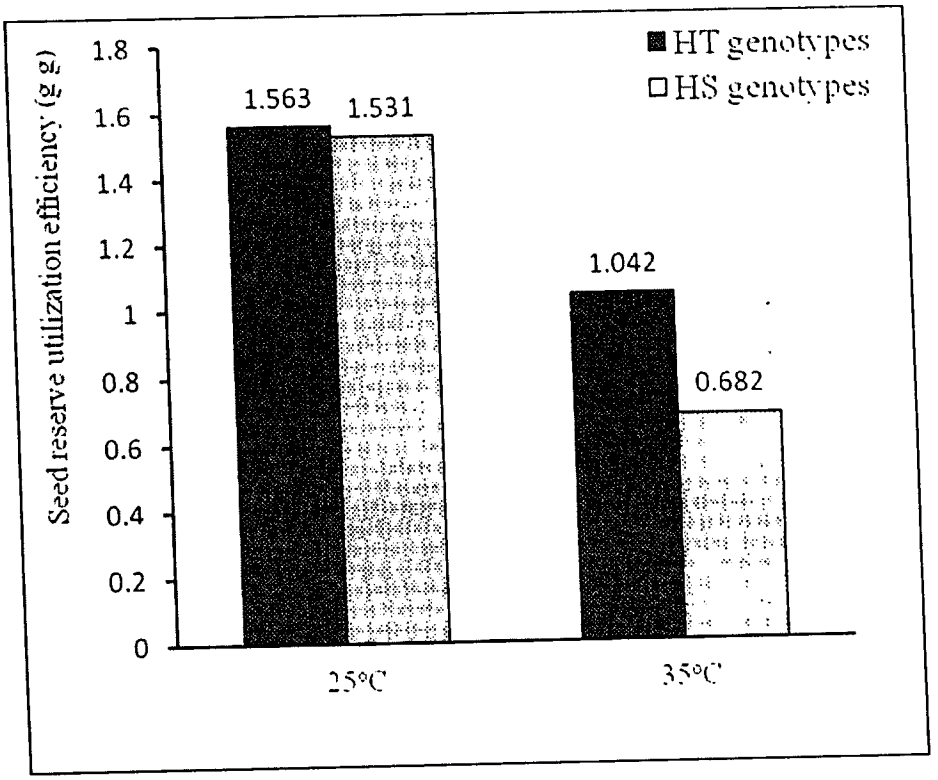


Fig. 3.4. Average seed reserve utilization efficiency (g/g) of heat tolerant and heat sensitive wheat genotypes as influenced by temperature regimes.

0.880 g/g). The HT wheat genotypes maintained a higher SRUE (1.042 g/g) than the HS genotypes (0.682 g/g) at high temperature stress though both groups of the wheat genotypes maintained a more or less same SRUE (1.531 g/g in HS and 1.563 in HT genotypes) under normal germination temperature (Fig. 3.4). The reduction in SRUE during germination from 25°C to 35°C is indicated by the relative value. The relative value of SRUE at 35°C compare to 25°C also showed that the reduction in SRUE at high temperature was higher in HS wheat genotypes than in HT genotypes.

Relationship between membrane injury (%) and seed reserve utilization efficiency

A significant negative correlation ($r = -0.78^{**}$, $n = 20$) was found across the wheat genotypes between membrane injury (%) and SRUE at 35°C (Fig. 3.6). Wheat genotypes with low SRUE at 35°C tended to show greater membrane injury. This association was insignificant ($r = 0.07^{NS}$, $n = 20$) between membrane injury (%) and SRUE at 25°C (Fig. 3.5).

Heat stress reduces the efficiency of conversion of endosperm mass into seedling tissues so that the mass of dark grown seedling was smaller under heat stress than under normal temperature. At high temperature, SRUE was reduced in all wheat genotypes, it may be due to the degree of reduction in seedling dry weight was greater but the degree of reduction in respiratory loss of seed reserve was lower at high temperature than under normal temperature. Seed reserve utilization efficiency under high temperature varied with genotypes, and heat tolerance in terms of SRUE is probably a result of lower loss of endosperm carbon to leaching and/or respiration as well as sustained conversion efficiency (Amthor and McCree, 1990). The size of carbon storage available at the onset of germination, as reflected in seed mass seems

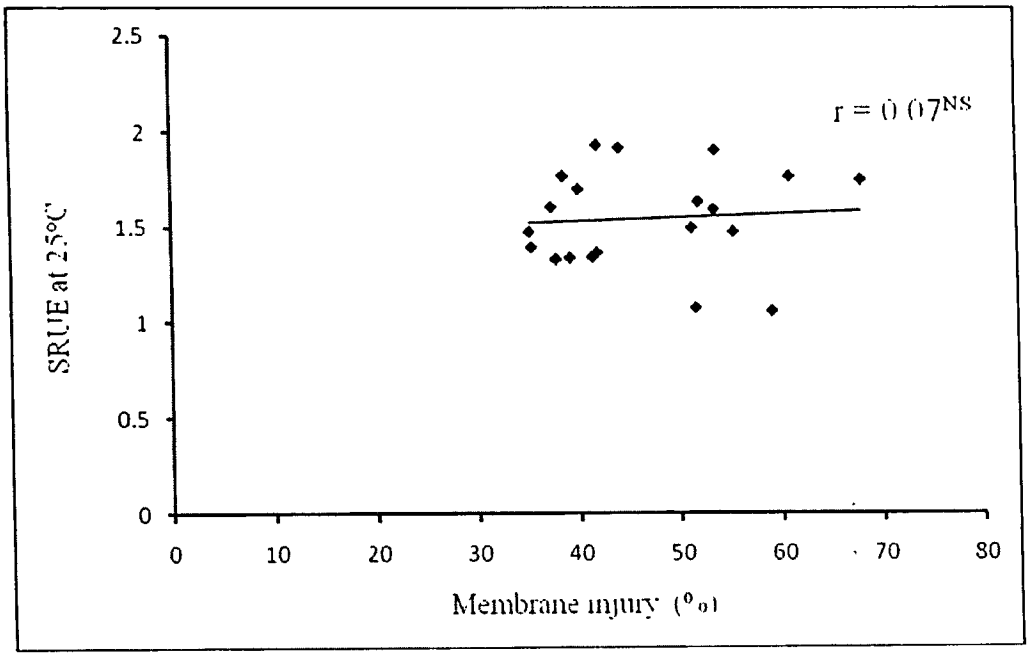


Fig. 3.5. Relationship ($r = 0.07^{NS}$, n= 20) between membrane injury (%) to high temperature of seedling and seed reserve utilization efficiency during germination at 25°C in twenty wheat genotypes.

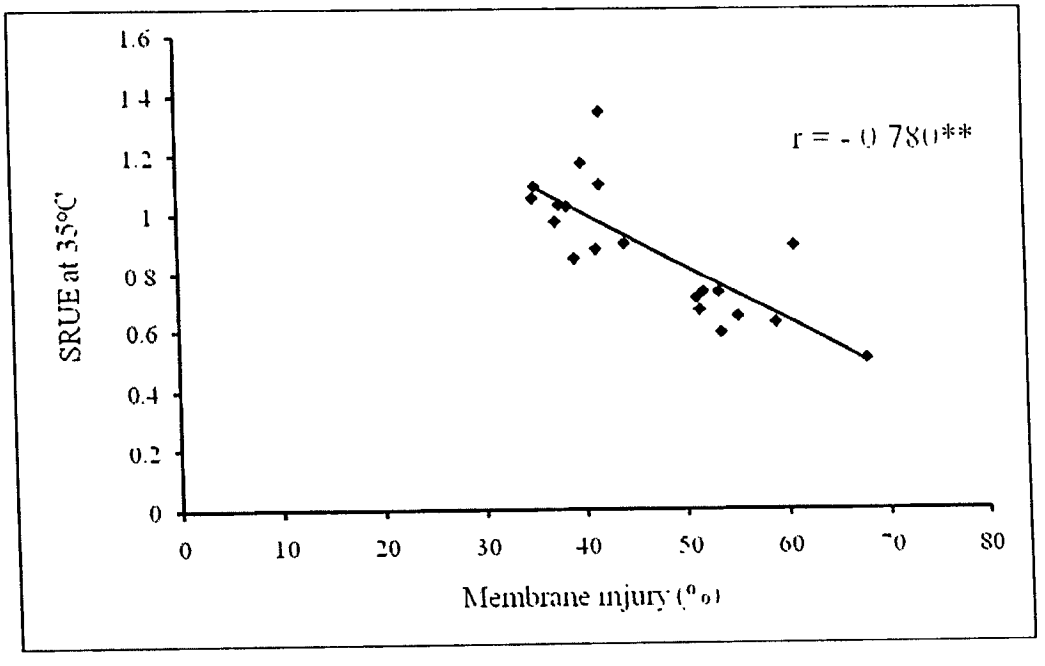


Fig. 3.6. Relationship (r = - 0.780**, n= 20) between membrane injury (%) to high temperature of seedling and seed reserve utilization efficiency during germination at 35°C in twenty wheat genotypes.

to have little relation to the variation in SRUE among wheat genotypes under heat stress because the remaining seed even contained higher amount of seed reserve under heat stress compared to normal temperature. Membrane thermostability of the autotrophic plant under heat stress is a reputed measure of heat tolerance in growing wheat plants. In this study, heat tolerance of the autotrophic growing plant in terms of membrane injury (%) was found to be well associated across wheat genotypes with heat tolerance in terms of SRUE. Hasan *et al*, (2004) and Blum & Sinmena (1994) also found heat tolerance in terms of SRUE in different wheat genotypes.

The results of the present study indicate that the seed reserve utilization efficiency during germination reduced due to high germination temperature compared to that at 25°C in all wheat genotypes tested but the reduction was lesser in HT wheat genotypes than that in HS wheat genotypes. The heat tolerance in terms of SRUE can be used to screen tolerant wheat genotypes against heat stress which is comparable to cell membrane thermostability test.

Experiment 2. EVALUATION OF SEEDLING PROLINE CONTENT OF WHEAT GENOTYPES IN RELATION TO HEAT TOLERANCE

INTRODUCTION

Proline is a non protein amino acid and compatible solute commonly accumulates in many plants exposed to various stress conditions such as water stress (Barnett & Naylor, 1966; Blum & Ebercon, 1976), salinity (Stewart & Lee, 1974; Treichel, 1975), air pollution (Godzic & Linskens, 1974) and unfavourable temperature (Chu *et al.*, 1974; Chu *et al.*, 1978). Under stresss condition, proline is synthesized from glutamate due to loss of feedback regulation in the proline biosynthetic pathway (Boggess and Stewart, 1980). This biosynthesis might be an adaptive mechanism to reduce the accumulation of NADPH, which increased as result of the decrease in photosynthetic CO_2 reduction rate of the plant (Berry and Bjorkman, 1980). Proline has also been found to serve as a substrate for respiration (Britikov *et al.*, 1965) and as a source of nitrogen and other metabolites (Stewart and Boggess, 1978).

Genotypic variations in proline accumulation have been observed in many studies and attempts were made to correlate proline accumulation with tolerance of plants to stress. This apparent correlation between proline accumulation and environmental stress suggests that proline could have a protective function. Recently, Paleg *et al.*, (1981) demonstrated that a number of solutes, including proline, protected enzymes, isolated from various tissues, from inactivation by heat. Rapid catabolism of proline upon relief of stress may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress and repair of stress induced damage (Hare and Cress, 1997). Proline accumulation in high temperature has been reported in barley and radish leaves (Chu *et al.*, 1974), in tomato floral buds and leaves (Kuo *et al.*, 1986) 1.5 fold high in mulberry leaves

(Chaitanya, 2001), in the leaves of Brassica vegetables (Takeda, 1999), in cotton leaves (Ronde *et al.*, 2001), in cabbage and Chinese cabbage (Hossain *et al.*, 1995), in apple (Park *et al.*, 2001) and in flag leaves of wheat (Hasan *et al.*, 2007). Under supraoptimal temperature genotypic difference in proline accumulation pattern has also been reported in six cotton cultivars (Ronde *et al.*, 2001) and in different cabbage and Chinese cabbage varieties (Hossain *et al.*, 1995). Hasan *et al.* (2007) reported genotypic difference in change in flag leaf and kernel proline level in heat tolerant and heat sensitive wheat genotypes due to post anthesis heat stress condition. But in wheat, the heat tolerance in relation to seedling proline content was not yet evaluated. Therefore, the objectives of the present study were-

- to evaluate the effect of high temperature stress on proline accumulation in twenty wheat genotypes and
- to analyse the suitability of proline accumulation as a heat tolerance index of wheat.

MATERIALS AND METHODS

Location and duration

The experiment was conducted at Crop Botany Laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during the period from July to November 2006.

Seedling establishment

Seeds of 20 wheat genotypes used in the experiment-1 were geminated in plastic tray (50 × 35 × 8 cm) filled with soil and allowed to grow in Phytotron (NK SYSTEM BIOTRON, Osaka Nippon Medical & Chemical Instrument Co. Ltd. Tokyo, Japan, Model No. LPH-200-RD) with 16 h photoperiod, a light intensity of 200 uE $m^{-2}s^{-1}$, relative humidity of 90±1(%) and at about 25 °C for 8 days. Out of six sets three sets of seedlings (three replications) were allowed to grow in the same condition for another 48 hours for determination of seedling proline content under normal condition. Another three sets of seedlings were exposed to high temperature stress at 35 °C for 48 hours. The seedlings were then used for measuring membrane injury (%) and also for determination of seedling proline content.

Membrane thermal stability test

Procedure used for measuring membrane injury to high temperature was the same as describe by Blum and Ebercon (1981). Three samples per genotype were taken for the MT test to provide experimental replication, in a completely randomized design. Samples collected from each experimental unit consisted of paired sets (control and heat treated) of 10 leaf segments (4 cm long) cut from randomly selected first leaf. Paired sets of leaf segments were collected in separate test tubes. Leaf segments were washed thoroughly with three changes of deionized water to remove electrolytes

adhering to leaf tissue, as well as electrolytes released from cut ends of the leaf segments. After rinsing, tubes were drained, retaining 4 ml of water to prevent desiccation of tissue during heat treatment. For heat treatment test tubes were covered with aluminum foil and incubated in a controlled temperature water bath (J. P. SELECTA, Ctra-Nil-Km- 585.1, Abrera, SPAIN) at 44 °C for one hour, while control test tubes were maintained at 25°C during the same period of time. After the treatment period, 16 ml of deionized water was added to both control and treated test tubes. Test tubes were held at 10°C for 24 hours to allow diffusion of electrolytes from the leaf segments. Then the test tubes were brought to 25°C and shaken to mix the contents. An initial conductance of the test tube contents was determined with an electrical conductivity meter (Model HI 8083, HANNA instruments, Portugal). The test tubes were then placed in an autoclave at 121°C and 0.10 MPa pressure for 10 minutes to kill leaf tissue completely and release all of the electrolytes. Subsequently, test tubes were cooled to 25°C, the contents were mixed and a final conductance measurement was made. The level of injury was determined as relative injury (RI) from the following equation-

$$RI(\%) = 1 - [\{1 - (T_1/T_2)\}/\{1-(C_1/C_2)\}] \times 100$$

Where T and C refer to conductance values for treatment and control test tubes, respectively, and subscripts 1 and 2 refer to initial and final conductance values, respectively. The data were analyzed in randomized complete block design and the means were separated by DMRT at 5% level.

Estimation of proline

Leaf segments (0.5g) from each replication of each genotype were taken for proline estimation. Subsequently proline was estimated as Bates (1973).

At first Ninhydrin reagent was prepared in such a way so that it was utilized for proline estimation within two hours of preparation. For preparing Ninhydrin reagent, addition of 30 ml glacial acetic acid and 20 ml 6M orthophosphoric acid was mixed with 1.25 g of Ninhydrin. It was subsequently heated and stirred gently to dissolve but the temperature was not allowed to exceed 70^0C. Proline standards were prepared for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/ml with distilled water.

0.5g of fresh sample was crushed in mortar and pastte and homogenized the material in 10 ml 3% sulphosalicylic acid until no large segments of plant material remained. Homogenate was filtered through Whatman No. 2 filter paper. Two ml of the filtrate and each standard proline solutions were then reacted with 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid in a pyrex test tube, boiled for one hour at 100^0C in water bath covering the tube with aluminium foil to prevent excess evaporation. Subsequently, it was cooled in ice bath and 4 ml of toluene was added to each tube using a dispensor. Each tube was then shaken vigorously for 15 to 20 seconds in an electrical shaker and allowed the layer to separate for 30 minutes. The absorbance of layer was measured through spectrophotometer at 520 nm with pure toluene as a blank. The proline content was determined from a standard curve and calculated on a freash weight basis as follows:

µmoles proline / g of fresh plant material = {(µg proline / ml × ml toluene) / 115.5 µg / µmoles} / (g sample/5)

Relative performance

The relative performance was calculated as Asana and Williams (1965) by the following formula-

$$\text{Relative performance (\%)} = \frac{\text{Variable measured under stress condition}}{\text{Variable measured under normal condition}} \times 100$$

Statistical analysis

The data were analyzed in two factor randomized complete block design and the means were separated by DMRT at 5% level. Correlation analysis was also done and level of significance was tested with t-test.

RESULTS AND DISCUSSION

In the present experiment the membrane injury to high temperature of 10 day old seedlings of twenty wheat genotypes was measured as describe by Blum and Ebercon (1981). The seedling proline content of those genotypes was also estimated as Bates (1973). The tolerance of wheat genotypes as measured by membrane thermostability test were compared with the tolerance in terms of seedling proline content. The results of the present experiment in the form of table and figures along with necessary discussion have been presented in this part.

Membrane injury to high temperature

Membrane injury(%) to high temperature of 10 days old seedling was varied significantly among twenty wheat genotypes, with the lowest value in IVT 9 (20.68%) & the highest value in Pavon 76 (68.97%) and a mean of 49.00% (Fig. 1). The variation in membrane injury (%) indicates a wide variation in heat tolerance among the wheat genotypes tested. The wheat genotypes which showed equal or greater than 50% membrane injury were- Shatabdi (61.96%), Prodip (56.46%), BAW 1064 (50.10%), Gourab (57.84%), Pavon 76 (68.97%), Sonora (66.70%), Kalyansona (66.26%) and IVT 6 (52.46%), and were considered as heat sensitive (HS) genotypes. The other twelve wheat genotypes i. e., Bijoy (41.85%), Sufi (42.14%), Kanchan (42.20%), Fang 60 (49.00%), BAW 1059 (48.85%), BL 1883 (40.00%), BL 1022 (35.43%), IVT 7 (35.53%), IVT 8 (37.60%), IVT 9 (20.68%), IVT 10 (49.07%) and BAW 917 (49.69%) showed less than 50% membrane injury in membrane thermostability test and were grouped as heat tolerant (HT) genotypes. The grouping of seventeen wheat genotypes out of twenty was consistent with the observations of

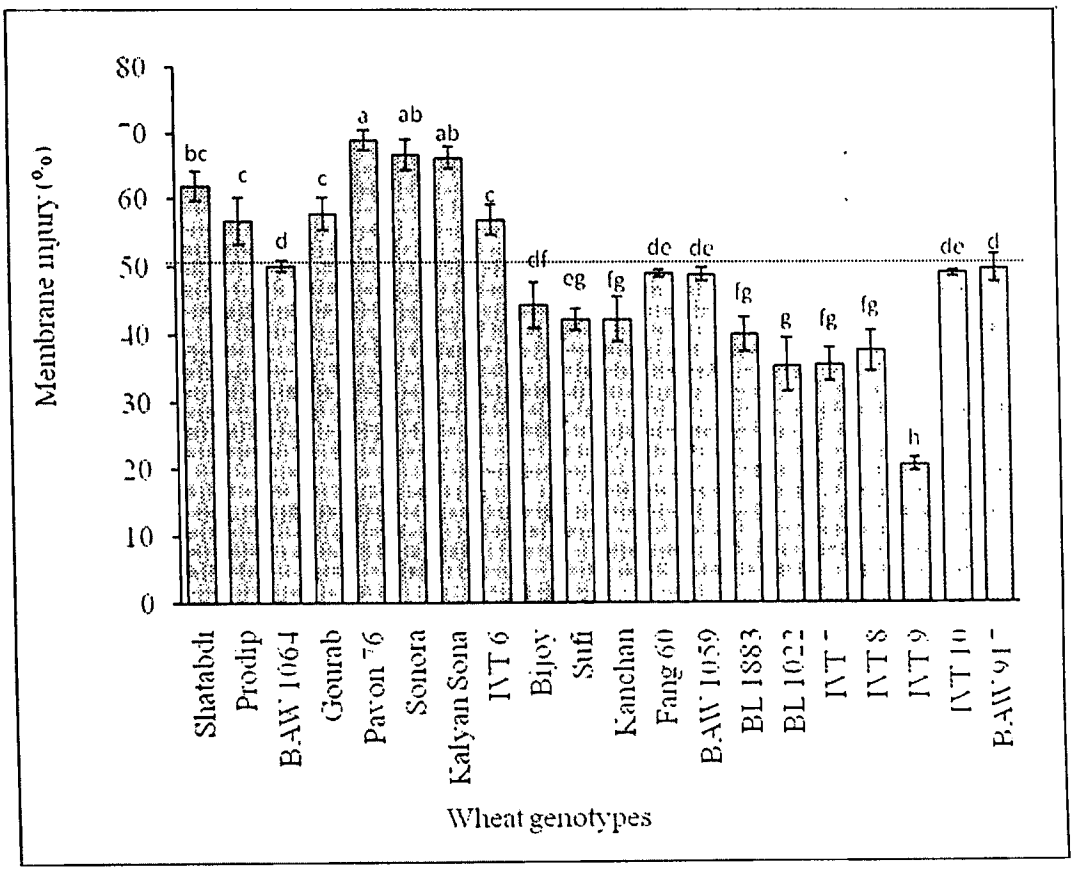


Fig. 4.1. Membrane injury (%) (mean ± SE) to high temperature of 10 days old seedling of 20 wheat genotypes measured.

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

the previous experiment. In the present study, wheat genotypes- Bijoy and Sufi laid in heat tolerant group though they were classified as heat sensitive in the previous experiment. On the other hand, IVT 6 was indicated as heat tolerant in the previous experiment though it was grouped as heat sensitive in the present study.

Seedling proline content

Proline content of 10 days seedling was influenced significantly by the interaction effect of temperature regimes and wheat genotypes (Table 4.1). Seedling proline content at 35°C, with a range of 1.03 µmoles/g FW (in Pavon 76) to 2.17 µmoles/g FW (in IVT 9) and an average of 1.786 µmoles/g FW, were higher compared to those at 25°C (with a range of 0.60 to 1.13 µmoles/g FW and an average of 0.891µmoles/g FW). The increments of seedling proline content from 25°C to 35°C were significant for all wheat genotypes tested. At 25°C, the seedling proline content was more or less same in heat sensitive (a mean of 0.88 µmoles/g FW) and heat tolerant (a mean of 0.902 µmoles/g FW) groups, but at 35°C, higher amount of proline was found in HT genotypes (a mean of 1.953 µmoles/g FW) compared to that in HS genotypes (a mean of 1.535 µmoles/g FW) (Fig. 4.2).

The increment of proline level from 25°C to 35°C in different wheat genotypes is indicated by the relative value, The relative value indicated that at 35°C the HT genotypes produced more than double (>200%) proline than that at 25°C. On the other hand, the HS produced less than double (<200%) proline at 35°C compared to that at 25°C.

Table 4.1. Proline content (mean ± SE) in 10 days old seedling of twenty wheat genotypes as influenced by two temperature regimes

Genotypes	Seedling proline content (µmoles proline /g fresh weight)		
	At 25°C	At 35°C	Relative to 25°C (%)
Shatabdi	1.13 ±0.05 f	2.10 ±0.16 a	186
Prodip	0.82 ±0.05 gk	1.60 ± 0.05 d	195
BAW 1064	0.87 ±0.06 gi	1.71 ±0.07 cd	197
Gourab	0.95 ±0.06 fi	1.64 ±0.06 cd	173
Pavon 76	0.60 ±0.03 k	1.03 ±0.10 fh	172
Sonora	0.91 ±0.06 fi	1.36 ±0.14 e	149
Kalyan Sona	0.73 ±0.02 ik	1.14 ±0.06 f	156
IVT 6	1.06 ± 0.06fg	1.70 ±0.10 cd	160
Bijoy	0.96 ±0.06 fi	2.08 ±0.07 ab	217
Sufi	0.64 ±0.03 jk	1.68 ±0.05 cd	263
Kanchan	0.85 ±0.04 gi	1.86 ± 0.05 bc	219
Fang 60	0.80 ±0.06 hk	1.95 ±0.05 ab	244
BAW 1059	0.85 ±0.09 gj	2.12 ±0.09 a	249
Bl 1883	1.06 ±0.31 fg	1.50 ±0.05 de	142
BL 1022	0.98 ±0.11 fh	1.94 ±0.02 ab	198
IVT 7	0.92 ±0.05 fi	1.86 ±0.05 bc	202
IVT 8	0.91 ±0.05 fi	2.03 ±0.04 ab	223
IVT 9	0.87 ±0.07 gi	2.17 ±0.14 a	249
IVT 10	1.03 ±0.07 fh	2.15 ±0.09 a	209
BAW 917	0.95 ±0.07 fi	2.09 ±0.06 ab	220
Mean	0.891	1.786	-
CV (%)	9.42		-

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

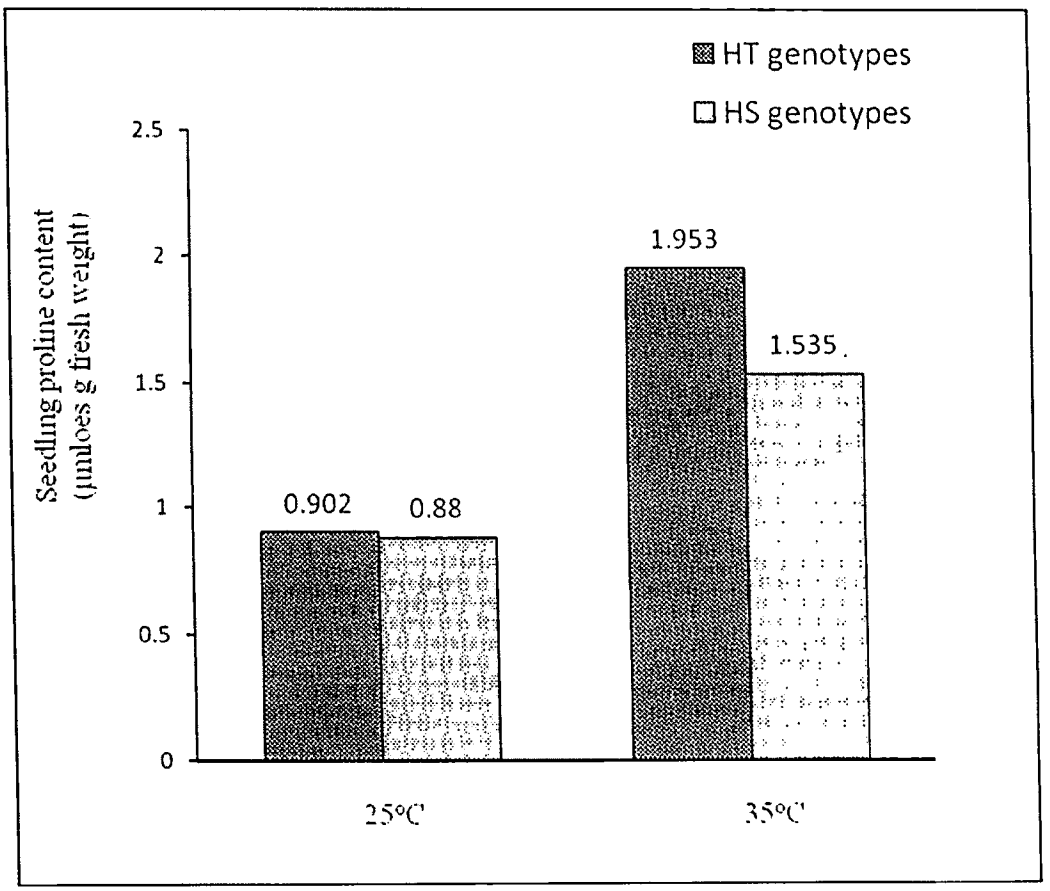


Fig. 4.2. Average seedling proline content of heat tolerant and heat sensitive wheat genotypes as influenced by temperature regimes.

Relationship between membrane injury (%) and seedling proline content

The seedling proline content at 35°C and membrane injury (%) maintained a significant negative correlation (r = -0.619**) across the twenty wheat genotypes (Fig. 4.4), indicating wheat genotypes with high proline level at 35°C tended to show greater thermotolerance. This association was insignificant (r = -0.155^{NS}) between proline content in seedling at 25°C and percent membrane injury (Fig. 4.3).

Under stresss condition, proline is synthesized from glutamate due to loss of feedback regulation in the proline biosynthetic pathway (Boggess and Stewart, 1980). Rapid catabolism of proline upoon relief of stress may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress and repair of stress induced damage (Hare and Cress, 1997). The relationship between proline accumulation and environmental stress suggests that proline could have some protective function. Paleg *et al.*, (1981) demonstrated that a number of solutes, including proline, protected enzymes, isolated from various tissues, from inactivation by heat. The accumulation of free proline may also contribute to the scavenging of heat stress induced active oxygen species by enhancing photochemical electron transport activities (Alia *et al.*, 1991). Under supraoptimal temperature genotypic difference in proline accumulation pattern has also been reported in six cotton cultivars (Ronde *et al.*, 2001), different cabbage and Chinese cabbage varieties (Hossain *et al.*, 1995). In the present study, heat tolerance of different wheat genotypes expressed as membrane injury (%) was found to be well associated with heat tolerance in term of proline content. Hasan *et al.* (2007) also found heat tolerance in terms of proline accumulation in flag leaf and kernel of different wheat genotypes.

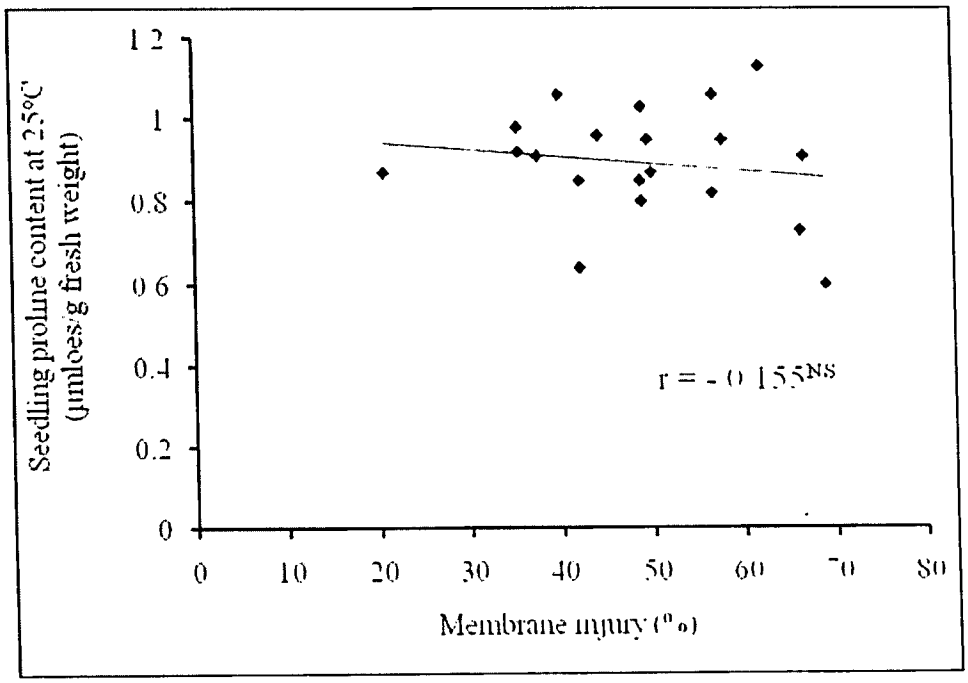


Fig. 4.3. Relationship ($r = -0.155^{NS}$, n = 20) between membrane injury (%) to high temperature and proline content of seedling at 25°C in twenty wheat genotypes.

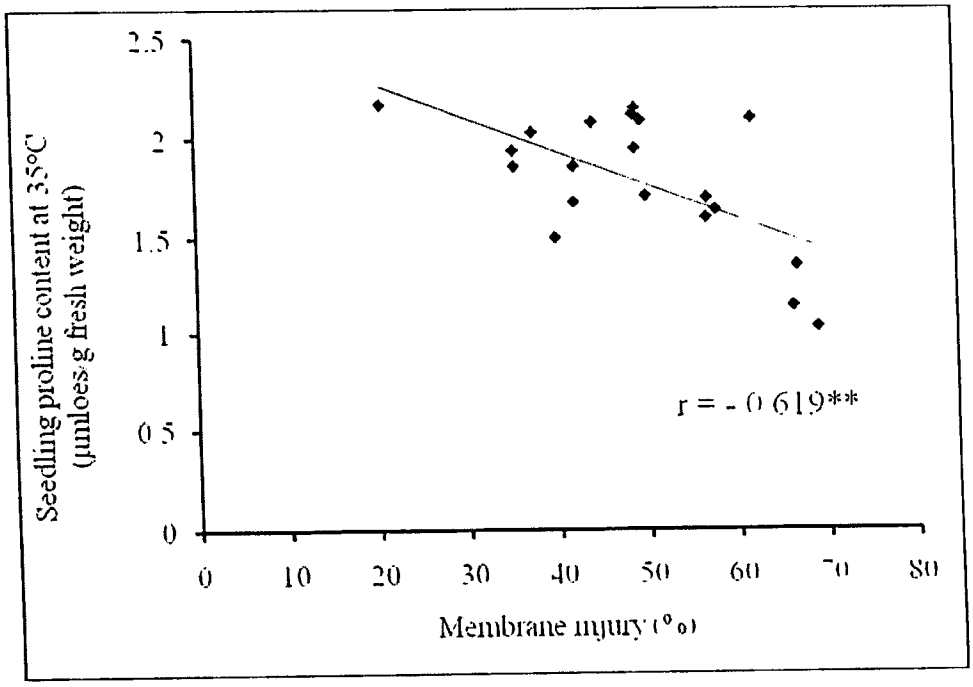


Fig. 4.4. Relationship ($r = -0.619^{**}$, n = 20) between membrane injury (%) to high temperature and proline content of seedling at 35°C in twenty wheat genotypes.

The ovaerall results of the present study indicate that the seedling of all wheat genotypes grown at 35°C maintained a higer proline level than those grown at 25°C. The increment in proline level was higher in HT wheat genotypes than that in HS genotypes. The increased seedling proline level due to high temperature can be used to screen heat tolerant wheat genotypes, which is comparable to cell membrane thermostability test.

Experiment 3. HEAT TOLERANCE AND ASSOCIATED PHYSIOLOGY OF WHEAT GENOTYPES DIFFERING IN MEMBRANE THERMOSTABILITY

INTRODUCTION

Productivity of wheat falls markedly at high temperature. High temperature stress after anthesis severely reduces reproductive development in wheat (Al- Khatib and Paulsen, 1984; Wardlaw *et al.*, 1980). Whether stress induced yield reduction is primarily due to diminished source or altered sink activity is often difficult to discern. It is apparent that stress reduces yield by decreasing source activity (Al- Khatib and Paulsen, 1984; Kuroyanagi and Paulsen, 1985; Spiertz, 1974), which is especially critical because most grain mass is from current photosynthesis after anthesis (Evans *et al.*, 1975). Substantial evidence also indicates that stress limits yield by reducing sink capacity for grain development (Dinar and Rudich, 1985; Nicolas *et al.*, 1984; Sofield *et al.*, 1977; Wardlaw *et al.*, 1980).

Source activity is damaged by heat because both leaf area duration (Herzog, 1982; Sofield *et al.*, 1977; Spiertz, 1974) and photosynthesis (Al- Khatib and Paulsen, 1984; Kuroyanagi and Paulsen, 1985) are reduced. Injury near PSII has frequently been identified in heat treated membranes (Laasch, 1987)) and intact tissues (Smillie and Hetherington, 1983). Heat injury limits sink growth potential particularly when stress is imposed during early sink developmental stages. At later developmental stages, stress reduces sink ability to absorb assimilates (Dinar and Rudich, 1985).

Source and sink activities are related by direct feedback mechanisms during normal condition (Plaut *et al.*, 1987). Effects of stress on feedback regulation depend partly on relative sensitivity of source and sink activities to the stress. Source and sink

interactions may be regulated to a common factor, e.g. cytokinin from roots, which influenced sink activity (Herzog, 1982; Kuroyanagi and Paulsen, 1988) and ability of chloroplast ultrastructure (Caers *et al.*, 1985). Heat injury to roots also may interfere with synthesis or transport of cytokonin and alter sink and source activity simultaneously (Kuroyanagi and Paulsen, 1988). Altered source activity or sink activity or combine effect of both due to heat stress may be the casual factors for a yield penalty through reduction in grain filling period as well as the rate of grain filling (Sofield, 1977, Hasan and Ahmed, 2005).

Proline is known to accumulate in flag leaves of wheat due to high temperature. Genotypic difference in change in flag leaf and kernel proline in heat tolerant and heat sensitive wheat genotypes has also been reported due to post anthesis heat stress condition (Hasan *et al.*, 2007). Recently, Paleg *et al.* (1981) demonstrated that a number of solutes, including proline, protected enzymes, isolated from various tissues, from inactivation by heat.

However, investigation about the physiological sensitivity in relation to source and sink activity would be helpful to take proper crop management to sustain yield under late planting heat stress condition. The present study was therefore under taken with the following objectives-

- to find out the proline accumulation pattern in flag leaf and kernel of wheat due to heat stress and
- to determine whether a genotype is source limiting or sink limiting for wheat yield in heat stressed environment.

MATERIALS AND METHODS

Location and duration

The experiment was carried out at the research farm (Plot No.14) of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during November, 2006 to Octobar, 2007 on an upland soil. It is located at the center of Madhupur Tract (24°05' N latitude and 90°25' E longitude) at an elevation of 8.4 m above the sea level.

Soil and climate

The soil of BSMRAU farm belongs to Sanla series of Shallow Red-Brown Terrace soil type with silty clay in surface and silty clay loam in sub-surface region. As per USDA soil classification, the experimental soil was under Ochrept sub-order of Inceptisol order. The information of basic physical and chemical properties of the soil are presented in Appendices 1and 2. The experimental site is situated in the sub-tropical region characterized by heavy rainfall during the months from May to September and scantly rainfall in the rest of the year. Meteorological data on rainfall, temperature, relative humidity and evaporation from December, 2006 to April, 2007 obtained from meteorological station of Plant Physiology Division, BRRI, Gazipur are presented in Appendix 3.

Experimental design and treatment

The experiment was conducted in a split plot design with three replications. The unit plot size was 3 m x 2 m having a plot to plot and block to block distance of 0.5 m. The distance between the main plots was 1.0 m. The two growing conditions- normal growing condition (sowing on November 30) and post anthesis heat stress condition (sowing on December 30) were placed in the main plots as main plot treatments

whereas six wheat genotypes selected from experiment 1 and 2 were placed randomly in the sub-plots as sub-plot treatments. Four heat tolerant (Kanchan, Fang 60, BAW 1059 and BL 1022) and two heat sensitive (Pavon 76 and Sonora) wheat genotypes based on membrane injury (%), seed reserve utilization efficiency and seedling proline level were selected for the present experiment.

The treatment factors A and B were-

Main plot treatment: Two growing conditions

1. Normal growing condition (sowing at November 30)
2. Post anthesis heat stress condition (sowing at December 30)

Sub-plot treatment: Six wheat genotypes

Kanchan
Fang 60
BAW 1059
BL 1022
Pavon 76
Sonora

Land preparation

The land was ploughed by a tractor drawn disc plough and was leveled by harrowing and laddering carefully. The weeds and stubble were removed and main plots, sub-plots and blocks were prepared.

Fertilizer application and irrigation

A fertilizer dose of 140-35-75-18-2-0.5 kg/ha N, P, K, S, Zn and B was applied in the form of Urea, Triple Supper phosphate (TSP), Murate of potash (MP), Gypsum, Zinc sulphate and Boric acid, respectively. After land preparation, full dose of P, K, S, Zn and B and one third of N were incorporated thoroughly into the soil as basal dose. The remaining amount of N was applied at 25 and 40 days after seedlings emergence

splited into two equal amounts (Anonymous, 2005a). Irrigation was given to avoid the drought stress when necessary.

Sowing

Seeds of six wheat genotypes (Kanchan, Fang 60, BAW 1059, BL 1022, Pavon 76 and Sonora) were sown on November 30 and December 30, 2007 in rows of 20 cm apart, at the rate of 120 kg/ha. November 30 sowing was regarded as normal and December 30 sowing as post anthesis heat stress growing conditions. After sowing slight irrigation with overhead sprinkler was given for uniform germination.

Intercultural operations

After sowing, care was taken against birds up to 15 days. The crop was kept weed free and for controlling diseases, Tilt 250 EC was sprayed regularly at 15 days interval after 30 days of sowing.

Membrane thermal stability (MT) test

Procedure used for measuring Membrane injury (%) to high temperature was the same as describe by Blum and Ebercon (1981). Flag leaf samples were collected at anthesis from five randomly selected plants of each genotypes and replication. Chen *et al.* (1982) indicated that it is important to provide a exposure to sub-lethal high temperatures prior to evaluation of heat tolerance by MT test. So, MT test was conducted from the flag leaves obtained from December 30 sowing. Two leaf discs (10 mm in diameter) were collected from a flag leaf using a leaf puncher (CAT 162 model, Ronnd Open Industry Co. Ltd., Japan). Samples collected from each experimental unit consisted of paired sets (control and heat treated) of 10 leaf discs. Paired sets of leaf discs were collected in separate test tubes (25 mm x 200 mm). Leaf discs were washed thoroughly with three changes of deionized water to remove

electrolytes adhering to leaf tissue, as well as electrolytes released from cut ends of the leaf segments. After rinsing, tubes were drained, retaining 4 ml of water to prevent desiccation of tissue during heat treatment. For heat treatment test tubes were covered with aluminum foil and incubated in a controlled temperature water bath (J. P. SELECTA, Ctra-Nil-Km- 585.1, Abrera, SPAIN) at 44 °C for one hour, while control test tubes were maintained at 25 °C during the same period of time. After the treatment period, 16 ml of deionized water was added to both control and treated test tubes. Test tubes were held at 10 °C for 24 hours to allow diffusion of electrolytes from the leaf segments. Then the test tubes were brought to 25 °C and shaken to mix the contents. An initial conductance of the test tube contents was determined with an electrical conductivity meter (Model HI 8083, HANNA instruments, Portugal). The test tubes were then placed in an autoclave at 0.10 MPa pressure for 10 minutes to kill leaf tissue completely and release all of the electrolytes. Subsequently, test tubes were cooled to 25 °C, the contents were mixed and a final conductance measurement was made. The level of injury was determined as relative injury (RI) from the following equation-

$$RI(\%) = 1 - [\{1 - (T_1/T_2)\}/\{1-(C_1/C_2)\}] \times 100$$

Where T and C refer to conductance values for treatment and control test tubes, respectively, and subscripts 1 and 2 refer to initial and final conductance values, respectively. The data were analyzed in randomized complete block design and the means were separated by DMRT at 5% level.

SPAD value

Five main shoots were selected from each plot at the time of anthesis and tagged. SPAD value was taken from middle portion of the flag leaf of the tagged main shoot at 2 days intervals from anthesis to senescence using Minolta chlorophyll meter

(Model: SPAD-502, Minolta Co. Ltd., Japan). Measurements at each experimental plot consisted average of five readings.

Kernel growth rate and stem reserve utilization

At anthesis 60 main shoots were tagged from each plot. Four tagged main shoots were harvested at every 4th day beginning from anthesis to quantify grain growth and stem reserve utilization. The harvesting of main shoots in all genotypes was continued up to 44 days after anthesis (DAA) for normal growing condition (November 30 sowing) and 40 DAA for post anthesis heat stress conditions (December 30 sowing). The harvested main shoots were separated in to ears, flag leaves and stem with leaf sheath. The ears, flag leaves and stem were kept in oven at 70^{0}C for 72 hours. After oven drying ears and stems were weighted with an analytical balance (AND Electronic Balance Model ER 180A A & D Company Limited, Tokyo, Japan). 20 grains of each genotype were separated from the middle two spikelets of every five ear. During separation only first and second grain of a spikelet were collected. Then weight of 20 grains of each genotype was taken with analytical balance. The samples were stored in a desiccator to avoid moisture absorption for sugar and starch analysis.

The absolute grain growth rate (AGR) was calculated using the following formula-

$$AGR = \frac{W_2 - W_1}{T_2 - T_1}$$

Where,

W_1 = Grain dry weight at initial time
W_2 = Grain dry weight at final time
T_1 = Initial time
T_2 = Final time.

Stem reserve utilization (g/ main shoot) was calculated using the formula from the model shown in figure 5.1.

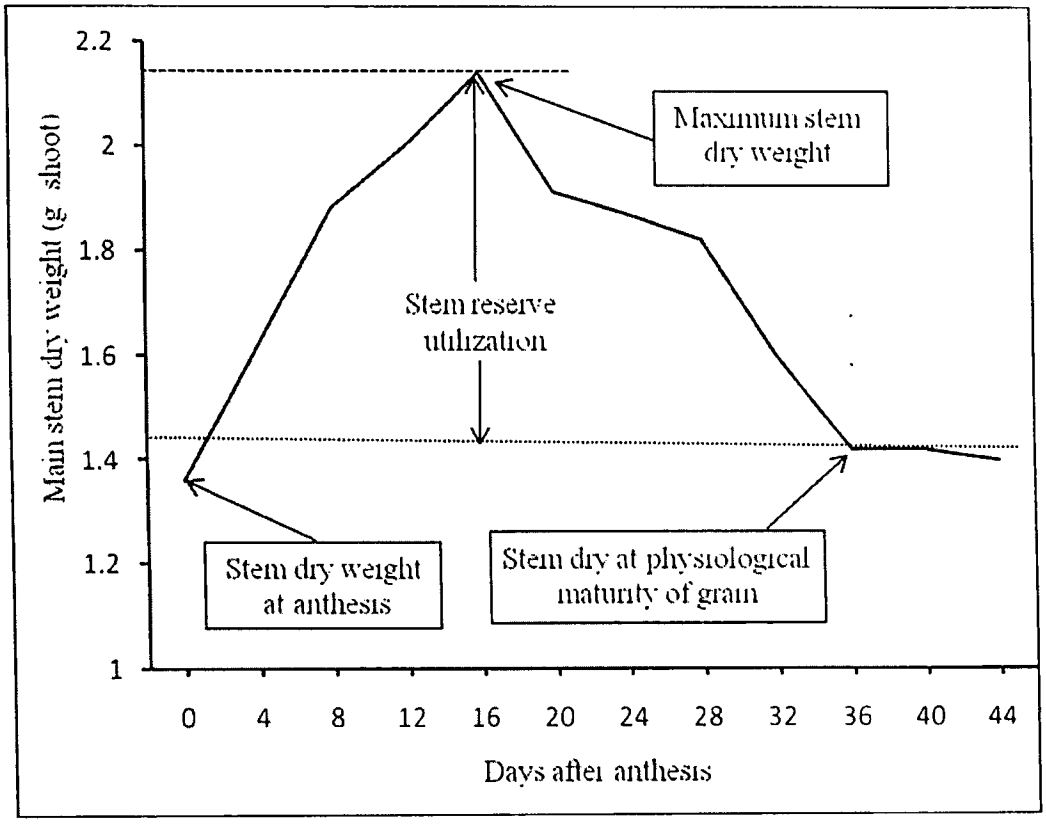


Fig. 5.1. Model for calculating stem reserve utilization (g/main stem).

Stem reserve utilization (g / main stem) =

Maximum stem dry weight – Stem dry weight at physiological maturity of grain

Grain dry weight/ main stem ear was calculated using the following formula-

Grain dry weight/ ear = Maximum ear dry weight – Ear dry weight at anthesis

Estimation of proline

Proline content of flag leaf and kernel at 8, 16 and 24 days after anthesis in all wheat genotypes grown in two different growing conditions were estimated. Flag leaves and

ear from each replication of each genotype were collected and immediately kept in the ice-bag and brought to Crop Botany Laboratory of BSMRAU for proline estimation. The kernels from ear were separated and 0.5g of fresh weight of both flag leaf and kernels were taken for proline estimation. Subsequently proline was estimated as Bates (1973).

At first Ninhydrin reagent was prepared in such a way so that it was utilized for proline estimation within two hours of preparation. For preparing Ninhydrin reagent, addition of 30 ml glacial acetic acid and 20 ml 6M orthophosphoric acid was mixed with 1.25 g of Ninhydrin. It was subsequently heated and stirred gently to dissolve but the temperature was not allowed to exceed 70°C. Proline standards were prepared for 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 µg/ ml with distilled water.

0.5g of fresh sample was crushed in mortar and pastte and homogenized the material in 10 ml 3% sulphosalicylic acid until no large segments of plant material remained. Homogenate was filtered through Whatman No. 2 filter paper. Two ml of the filtrate and each standard proline solutions were then reacted with 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid in a pyrex test tube, boiled for one hour at 100°C in water bath covering the tube with aluminium foil to prevent excess evaporation. Subsequently, it was cooled in ice bath and 4 ml of toluene was added to each tube using a dispensor. Each tube was then shaken vigorously for 15 to 20 seconds in an electrical shaker and allowed the layer to separate for 30 minutes. The absorbance of layer was measured through spectrophotometer at 520 nm with pure toluene as a blank. The proline content was determined from a standard curve and calculated on a freash weight basis as follows:

$$\text{µmoles proline / g of fresh plant material} = \{(\text{µg proline / ml} \times \text{ml toluene}) / 115.5\ \text{µg / µmoles}\} / (\text{g sample}/5)$$

Estimation of soluble sugar and starch

The soluble sugar and starch contents of flag leaf and kernel at 4 days interval from 12 days after anthesis to maturity in case of all wheat genotypes in both growing conditions were determined as Yoshida *et al.* (1976).

Sugar estimation: The dried composite flag leaf and kernel samples from four replicates were ground finely in mortar and pastel. 50 mg of finely ground sample was placed into a 15 ml centrifuge tube and 10 ml of 80% ethanol was added. The tubes were covered with aluminium foil and were kept in a water bath at 80-85^{0}C for 30 minutes. Then the samples were centrifuged and decanted into a 50ml beaker. This extraction was repeated three more times. The alcohol extract was evaporated on a water bath at 80-85^{0}C until most of the alcohol was removed (e.g. the volume was reduced to about 3 ml). The volume was made up to 25 ml with distilled water.

5ml of sugar extract was transferred to a 100-ml volumetric flask and made up to volume with distilled water. 5ml of this diluted sugar extract was taken into a pyrex test tube and then these tubes and the tubes containing the glucose standards (0, 0.1, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 mg/ 5ml) were put into an ice bath. To each tube 10 ml of anthrone reagent (2g of anthrone was dissolved in one liter of concentrated sulfuric acid and was stored in a refrigerator. A fresh solution was prepared every 2 days) was added slowly, allowing the reagent to run down the side of the test tube. The solution was stirred slowly with a glass rod. The tubes were put into boiling water bath for exactly 7.5 minutes and were cooled immediately in ice. Then the absorbance was measured at 630 nm and sugar content as mg/g dry weight was estimated using standard curve.

Starch estimation: The residue left in the centrifuge tube after collecting sugar extract was dried in an oven at 80^0C and 2 ml of distilled water was added to each centrifuge tube. The tubes were put in a boiling water bath for 15 minutes and stirred occasionally. The tubes were allowed to cool and 2ml of 9.2 N $HClO_4$ was added to each tube while stirring constantly. Then the solution was stirred occasionally for 15 minutes. The suspension was then made up to about 10 ml and centrifuged. The supernatant was collected and 2 ml of 4.6 N $HClO_4$ was added to the residue. This suspension was stirred for 15 minuets and was made up to 10 ml with distilled water. Then the suspension was centrifuged and the supernatants were combined. The combined supernatants were made up to 50 ml with distilled water.

5 ml of starch extract was transferred to a 50-ml volumetric flask and made up to volume with distilled water. 5 ml of this diluted starch extract was taken into a pyrex test tube. Then these tubes and the tubes containing glucose standards (0, 1, 2, 3, 4, and 5 mg/5ml including 0.6 ml 0.46 N $HClO_4$ solution for each tube) were put into ice bath. To each tube 10 ml of the anthrone reagent was added slowly allowing the reagent to run down the side of the test tube. The solution was stirred slowly with a glass rod. Then tubes were put in a boiling water bath for exactly 7.5 minutes and were cooled immediately in ice. Then the absorbance was measured at 630 nm and starch content was estimated as mg/g dry weight using standard curve.

Plant height

Plant height was measured from the base of the plant to the tip of the spike excluding the awn. Ten plants sample from each treatment were taken and means were calculated.

Spike character

Ten spikes from the main shoot were collected randomly per plot to collect spike characters data. Spike length (excluding awn) was measured manually using scale and number of kernels per spike was counted manually.

Number of spikes, grain and straw dry wt/m^2

The samples were collected from an area of 2m X 1m from the center of each plots by cutting the plant at ground level. Then ears were counted and collected in a cloth bag (2′ X 1.5′). The samples were dried in sun, threshed and cleaned manually and fresh weight of grain was taken. The husk, straw and representative samples of grain were dried in an oven at 70°C for 72 hours to obtain grain and straw dry weight. Total dry weight/m^2 was calculated by adding grain, husk and straw dry weight.

Individual kernel size

From each plot thousand grains were taken randomly from dried sample and weighed. From this weight, average individual kernel size was calculated.

Biological yield, straw yield and grain yield

The biological yield, straw yield and grain yield were expressed in t/ha, grain yield also adjusted to 12% moisture.

Relative performance

The relative performance was calculated as Asana and Williams (1965) by the following formula-

$$\text{Relative performance} = \frac{\text{Variable measured under stress condition}}{\text{Variable measured under normal condition}}$$

Heat susceptibility index

Heat susceptibility index (HSI) was calculated for grain yield as described by Fisher and Maurer (1978).

$$HSI = (1- Y/Y_p) / (1- X/X_p)$$

Where,

Y = Grain yield of genotype in a stress environment
Y_p = Grain yield of genotype in a stress-free environment
X = Mean Y of all genotypes
X_p = Mean X_p of all genotypes

(Higher HIS indicates greater susceptibility)

Statistical analysis

The findings were analyzed by partitioning the total variance with the help of computer by using MSTAT program. The treatment means were compared using Duncun's Multiple Range Test (DMRT) at 5% level of significance. Correlation analysis was also done and level of significance was tested with t-test.

RESULTS AND DISCUSION

A field experiment was conducted to study the influence of post anthesis hest stress on physiology related to source and sink activity in the heat tolerant and heat sensitive wheat genotypes and also on their yield attributes and yield. To expose the wheat genotypes in to normal growing temperature and natural heat stress during reproductive development, all wheat genotypes were sown in the field on November 30 and December 30. In this experiment, late sown (December 30) wheat genotypes exposed to heat stress (>26°C) during most of their reproductive growth phase and regarded as post anthesis heat stress condition. But the wheat genotypes sown on November 30 experienced less than 26°C throughout their whole reproductive growth phase and considered as normal growing condition (Fig. 5.2). Because, the optimum temperature for reproductive stage of wheat lies a range of 22 to 26°C (Campbell and Read, 1968). Temperature above 26°C at reproductive growth phase causes harmful premature ripening of wheat (Aborl *et al.*, 1991). Tashiro and Wardlaw, 1989 also found a reduction in grain growth duration and dry matter accumulation in grain in wheat with increasing temperature above a mean of 26.7°C. Temperatures above 25°C adversely affect flux through the pathway of starch synthesis in developing wheat and limit yield (Keeling *et al.*, 1994).

Results on heat stress induced changes in physiology related to source and sink activity of the heat tolerant and heat sensitive wheat genotypes and also their yield attributes and yield have been presented and discussed in the following paragraphs.

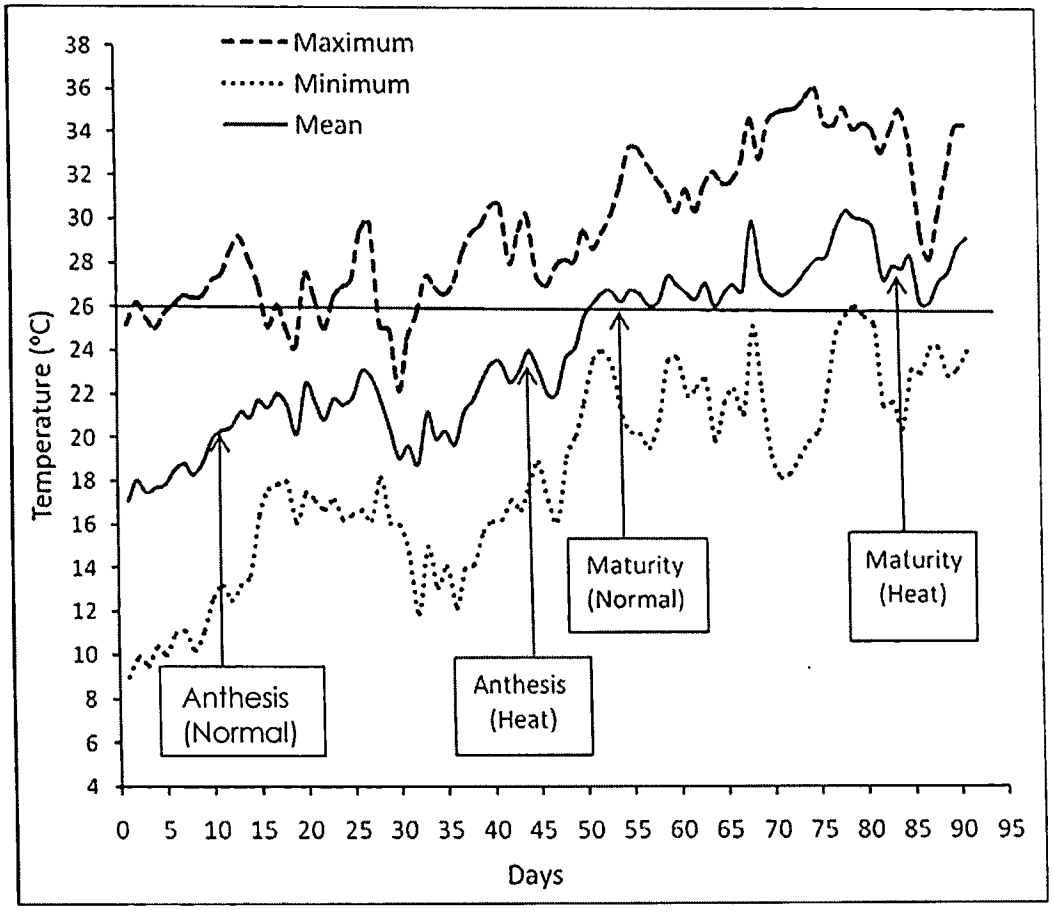


Fig. 5.2. Maximum, minimum and mean air temperature from 16 January to 16 April 2007 showing the period of anthesis and maturity of normal and late planting wheat.

Membrane injury to high temperature

Cell membrane thermostability of flag leaf at anthesis as expressed by relative membrane injury to high temperature differed significantly among the wheat genotypes tested (Fig. 5.3). The highest membrane injury was found in Pavon 76 (66.36%) which was followed by Sonora (60.02%) but they differed significantly between one another. BAW 1059 showed the lowest membrane injury (43.05%) which statistically identical with BL 1022 (43.78%). The membrane injury showed by Kanchan (48.84%) and Fang 60 (48.72%) were statistically similar and both of the genotypes were moderate based on relative membrane injury among the wheat genotyopes. Four heat tolerant wheat genotypes (Kanchan, Fang 60, BAW 1059 and Bl 1022) showed less than 50% membrane leakage and two heat sensitive genotypes showed more than 50% membrane leakage. So the result of the present study is consistent with the observations of the previous two experiments. Genotypic differences in membrane injury of flag leaf at anthesis of the field grown wheat was also reported by Hasan *et al.*, 2007, Mohi-Ud-Din *et al.*, 2007, Sikder *et al.*, 1999 and Shanahan *et al.*, 1990.

Flag leaf and kernel proline content

Flag leaf proline of different wheat genotypes at 8, 16 and 24 days after anthesis (DAA) was determined both under normal and heat stress condition and results were presented in figure 5.4. Under normal growing condition the heat tolerant genotypes contained 2.25 to 2.60 µmoles proline /g FW and the HS genotypes contained 2.66 to 4.1 µmoles proline /g FW in their flag leaves at 8 days after anthesis. Due to heat stress the proline level was increased in HT genotypes (2.68 to 3.54 µmoles/g FW) but in HS genotypes it was decreased (2.33 to 2.62 µmoles/g FW). At 16 days after anthesis, HT genotypes produced 2.6 to 3.42 µmoles proline /g FW and the HS

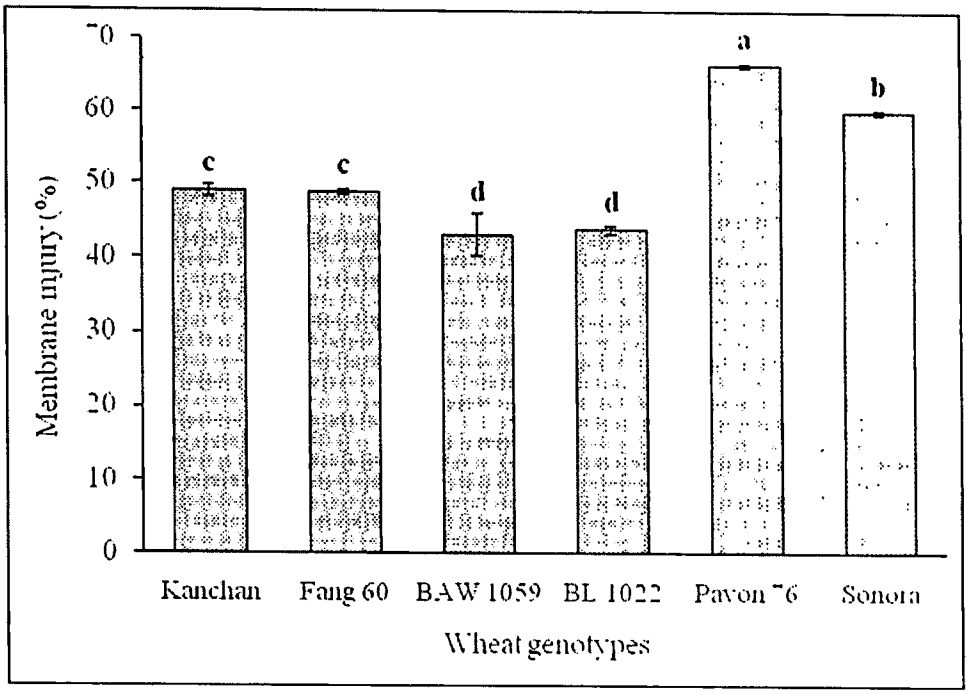


Fig. 5.3. Membrane injury (%) (mean ± SE) to high temperature of flag leaves (at anthesis) of different field grown wheat genotypes.

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

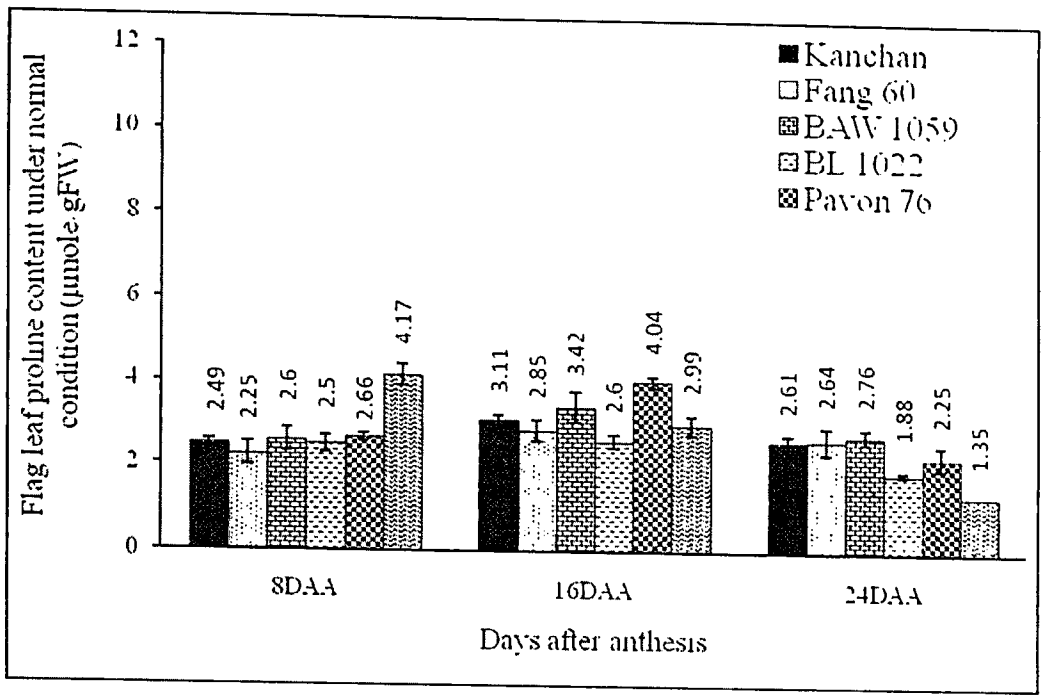
genotypes produced 2.99 to 4.04 µmoles proline /g FW under normal growing condition. This proline level increased many folds in HT genotypes (3.43 to 8.5 µmoles/g FW) but it was decreased in HS genotypes (2.33 to 2.74 µmoles/g FW) due to heat stress. Again at 24 days after anthesis, HT genotypes contained 1.88 to 2.76 µmoles proline /g FW under condition and it was increased due to heat stress (3.66 to 5.72 µmoles/g FW) but the flag leaves of HS genotypes contained more or less same proline both under normal (1.35 to 2.25 µmoles/g FW) and heat stress condition (1.84 to 2.11 µmoles/g FW). Overall results showed that both HT and HS genotypes maintained more or less similar proline level in their flag leaves throughout their whole reproductive growth period under normal condition. Due to post anthesis heat stress this proline level was increased in HT and decreased in HS genotypes, but the increment of proline level in HT genotypes were prominent at later reproductive growth period i.e. at 16 and 24 days after anthesis.

From both normal and heat stressed condition, kernel proline level was estimated at 8, 16 and 24 days after anthesis in different wheat genotypes and the results were presented in figure 5.5. Under normal growing condition, the kernel proline of HT genotypes was 2.42 to 3.66 µmoles/g FW and it was many folds higher in the kernel of HS genotypes (7.28 to 9.61 µmoles/g FW) at 8 days after anthesis. Due to heat stress the kernel proline level increased many folds in HT genotypes (5.29 to 9.3 µmoles/g FW) and it was decreased many folds in HS genotypes (2.1 to 4.2 µmoles/g FW). At 16 DAA, kernel proline level of all wheat genotypes showed more or less similar trend as shown at 8 DAA but the change in proline level due to heat stress was smaller. At 24 DAA, overall kernel proline level was higher under normal condition

than under heat stress condition and the changes in kernel proline due to heat stress were not distinct between HT and HS wheat genotypes.

Increased proline synthesis in heat stressed environment due to loss of feedback regulation in proline biosynthetic pathway (Boggess and Stewart, 1980) might be an adaptive mechanism to reduce the accumulation of NADPH, which increased as result of the decrease in photosynthetic CO_2 reduction rate of the plant (Berry and Bjorkman, 1980). Increased proline level both in flag leaf and kernel due to heat stress in HT wheat genotypes could have a protective function. Accumulation of free proline may contribute to the scavenging of active oxygen species by enhancing photochemical electron transport activities (Alia *et al.*, 1991). Proline may protect some enzymes from inactivation by heat (Paleg *et al.*, 1981). Rapid catabolism of proline upon relief of stress may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress and repair of stress induced damage (Hare and Cress, 1997). Genotypic difference in proline accumulation pattern due to heat stress has also been reported in six cotton cultivars (Ronde *et al.*, 2001) and different cabbage and Chinese cabbage varieties (Hossain *et al.*, 1995).

From the overall proline accumulation pattern in flag leaf and kernel, it was found that flag leaf and kernel maintained more or less similar proline level throughout the whole reproductive growth stage under normal condition. Due to heat stress, the proline level increased in kernel at early reproductive growth period but in flag leaf increased proline level was found during the later reproductive growth stage. This may be due to mobilization of proline between kernel and flag leaf under stressed condition. Kou *et al.* (1986) also suggested lower proline level in tomato leaves as a



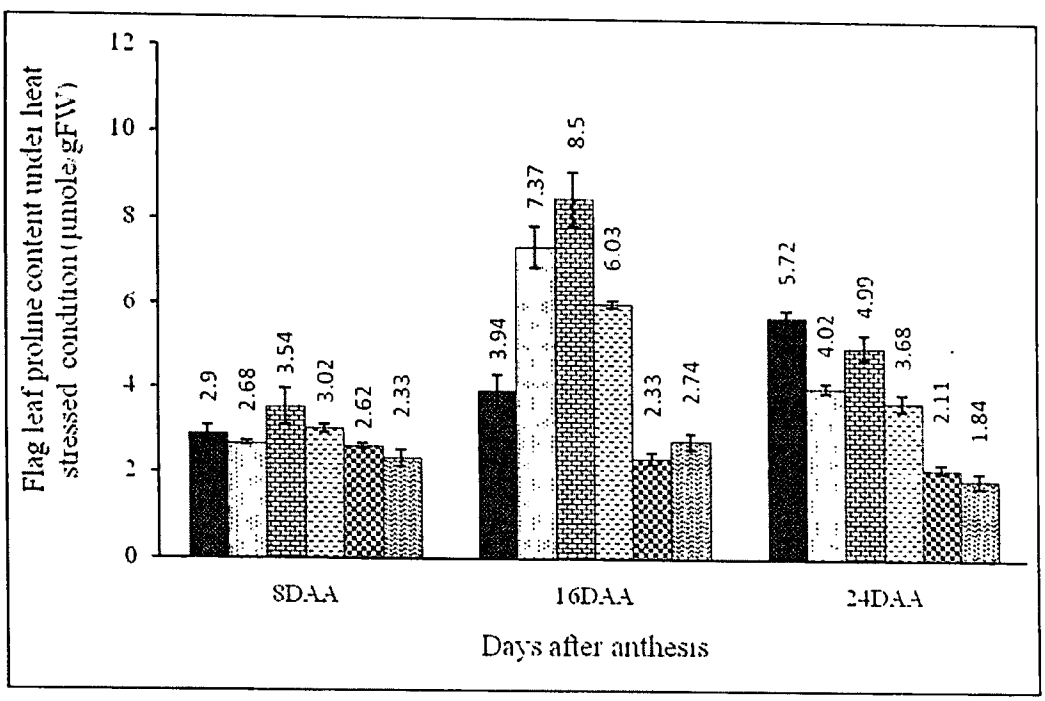


Fig. 5.4. Flag leaf proline content (mean ± SE) of different wheat genotypes at different days after anthesis as influenced by normal and heat stressed condition.

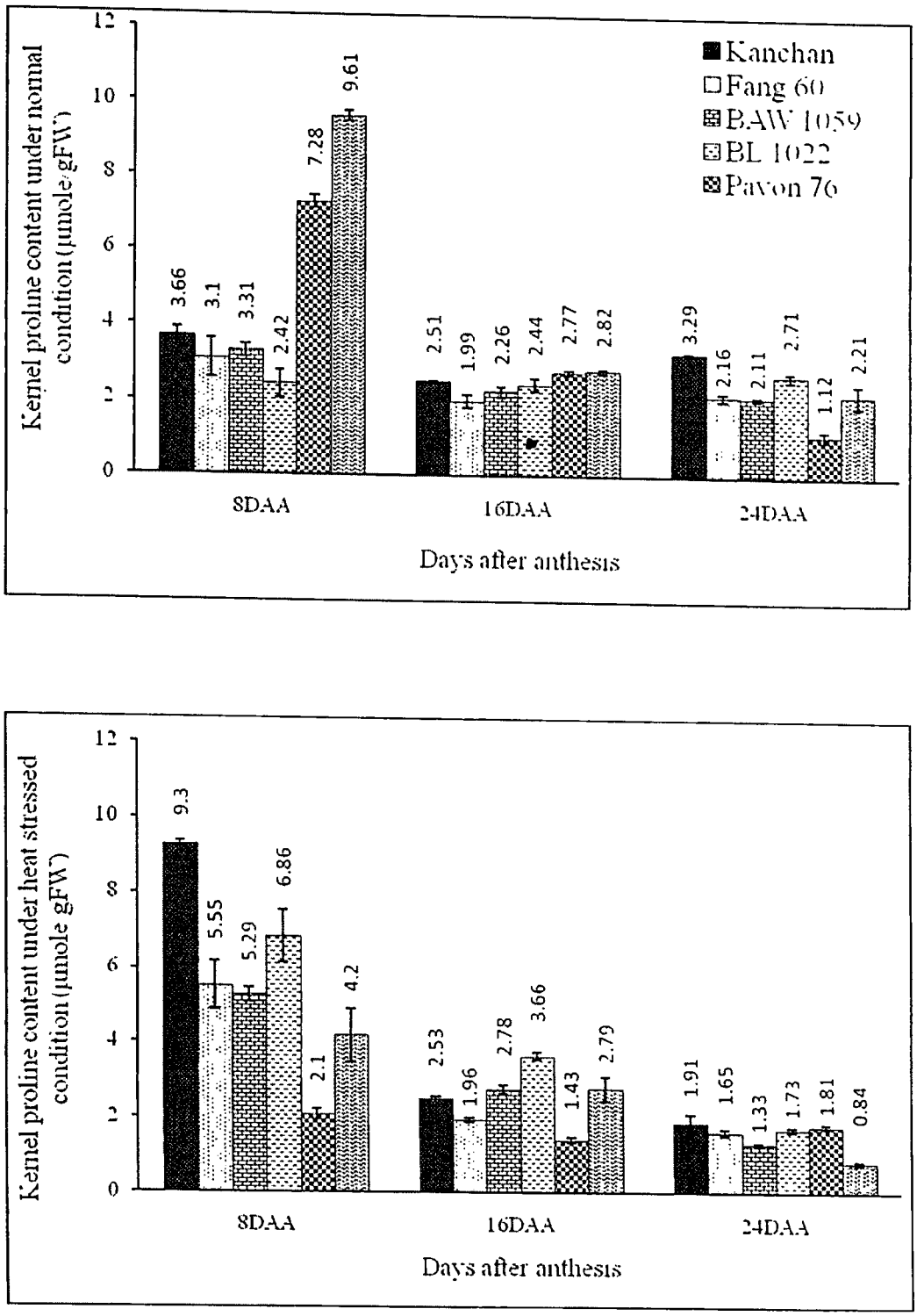


Fig. 5.5. Kernel proline content (mean ± SE) of different wheat genotypes at different days after anthesis as influenced by normal and heat stressed condition.

Table 5.1. Relationship between membrane injury (%) and relative proline content of flag leaf and kernel of different wheat genotypes at different days after anthesis (DAA) subjected to heat stress

Characters	DAA	Correlation of coefficient
Membrane injury (%) and relative flag leaf proline content	8	$r = -0.744^{NS}$
	16	r = - 0.877*
	24	r = - 0.843*
Membrane injury (%) and relative kernel proline content	8	r = - 0.853*
	16	r = - 0,860*
	24	$r = 0.590^{NS}$

result of higher proline accumulation in the floral buds. Distinct changes in kernel proline level between HT and HS wheat genotypes was found at early reproductive phase and in flag leaf, it was found at later reproductive growth stage due to heat stress. These results suggest that estimation of kernel proline at early reproductive stage and flag leaf proline at later reproductive growth stage may be suitable to measure the thermotolrance of wheat. In the present study, heat tolerance of different wheat genotypes expressed as membrane injury (%) was found to be well associated with heat tolerance in term of kernel proline content at 8 DAA (r = -0.853*) & at 16 DAA (r = -0.860*). This association was also well correlated with heat tolerance in terms of flag leaf proline content at 16 (r = -0.877*) and 24 (r = -0.843*) days after anthesis (Table 5.1). Hasan *et al.* (2007) also found heat tolerance in terms of proline accumulation in flag leaf and kernel of different wheat genotypes.

Flag leaf senescence

Senescence is an active, endogenously controlled degenerative process leading to death of whole plant or plant parts (Leopold, 1975). The exact start of senescence in wheat flag leaf is difficult to understand clearly in naked eyes. SPAD (Soil Plant Analysis Development) value has been well recognized as a mean of determining the onset of senescence process in a leaf (Rajcan *et al.*, 1999). The sharp decrease in SPAD value indicates the onset of leaf senescence. Rajcan *et al.* (1999) reported that leaf senescence of maize occurred at a SPAD reading between 25 and 30. Mohi-Ud-Din *et al.* (2007) found that the onset of flag leaf senescence of two wheat cultivars was indicated by the SPAD value reaching below 30. In the present study, sharp decrease in SPAD value which occurs at a SPAD reading in between 25 and 30 was used an indication of the onset of flag leaf senescence.

The SPAD value taken from the middle portion of flag leaf was the maximum at or near anthesis in all wheat genotypes both under normal and heat stress condition. The value was found to be decreased slowly during post anthesis stage and a rapid decrease in this value occurred at a SPAD reading in between 25 and 30 (Fig. 5.6).

Under normal growing condition, start of rapid decrease of SPAD value i. e. onset of flag leaf senescence occurred at 30 DAA in Kanchan, 32 DAA in Fang 60, 28 DAA in BAW 1059, 28 DAA in BL 1022, 26 DAA in Pavon 76 and 28 DAA in Sonora. Due to post anthesis heat stress, onset of flag leaf senescence occurred 8 day earlier in HT Kanchan, Fang 60, BAW 1059 and BL 1022. Onset of flag leaf senescence occurred 12 days earlier in heat sensitive Sonora. In another heat sensitive genotype, Pavon 76 rapid decrease in SPAD reading occurred only 4 days earlier due to heat stress (Table 5.2). The results of SPAD value showed that flag leaf longevity reducded in all wheat genotypes due to late planting heat stress but the reduction was inconsistent between HT and HS genotypes.

Possible reasons of early onset of flag leaf senescence due to terminal heat stress may be increased activity of peroxidase (Jiang *et al.*, 2007), increase in cholorophyll breakdown (Thomas *et al.*, 1980), Inactivation of oxygen evolving complex of PSII and chlophyll protein complex (Ferguson *et al*, 1993), inactivation of Rubisco (Grover *et al.*, 1986), reduction in chlorophyll biosynthesis, decreased level of cytokinin as a result of heat shock, loss of membrane integrity due to excessive fluidity of membrane lipid and denaturation of membrane bound H^+ pumping ATPase (Ranjan *et al.*, 2001). The heat tolerant wheat genotypes showed somewhat tolerance against heat stress induced flag leaf senescence. This may be due to the amelioration

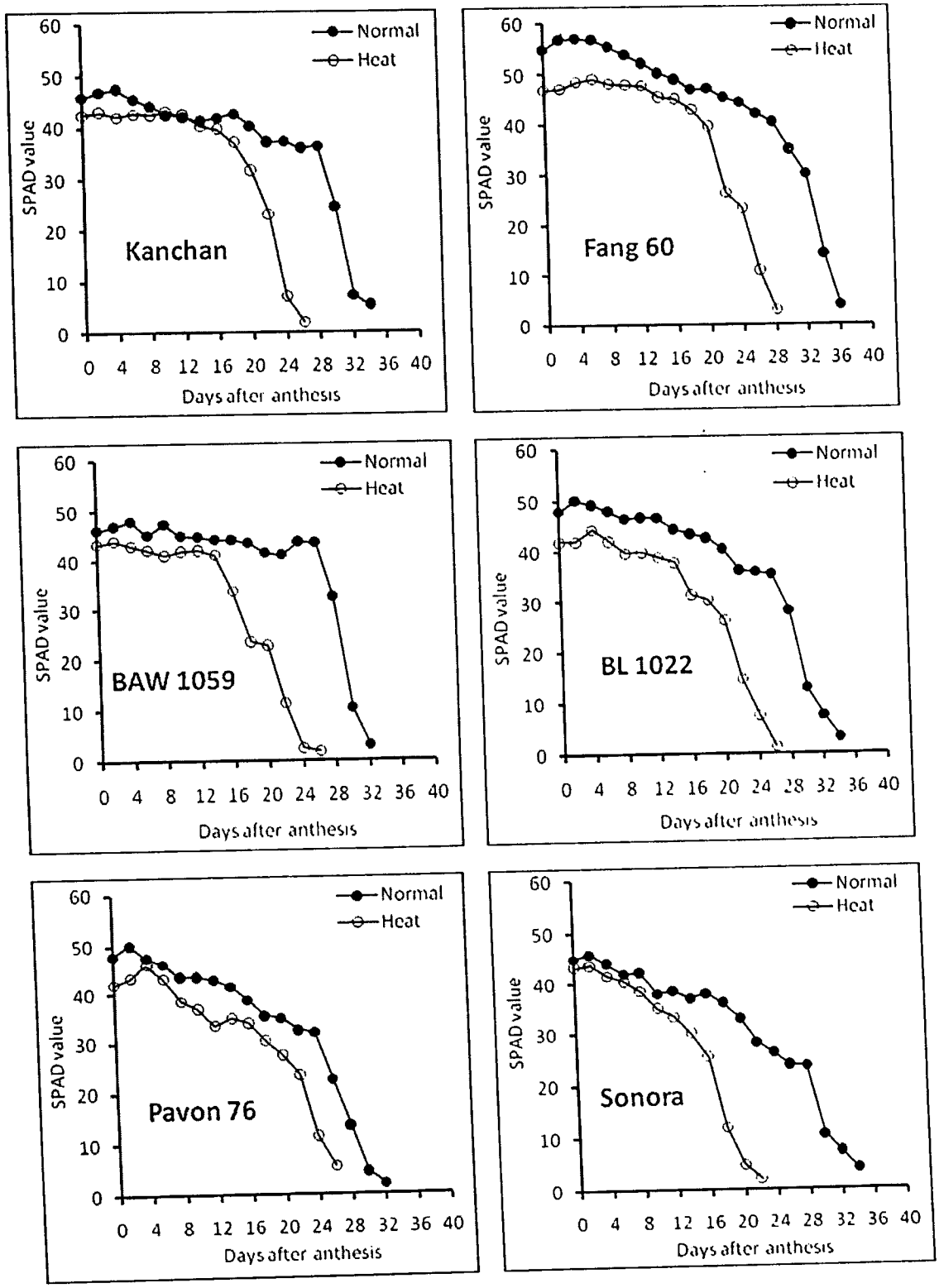


Fig. 5.6. SPAD value of different wheat genotypes at different days after anthesis as influenced by normal and heat stressed condition. (Each value is a mean of three replicates)

of high temperature induced oxidative stress by antioxidant enzymes like superoxide dismutase and catalase (Almeselmani *et al.*, 2006).

Ear dry matter accumulation

Both under normal and post anthesis heat stress conditions, a typical sigmoid pattern of dry matter accumulation in ear were discernible in all wheat genotypes (Fig. 5.7). Under normal growing condition, the ear dry weight in HT genotypes was observed to be increased up to 2.45 g in Kanchan, 2.59 g in Fang 60, 2.73 g in BAW 1059 and 2.52 g in BL 1022 at 36 days after anthesis and decline thereafter slowly. In two HS genotypes, the ear dry weight was found to be increased up to 2.21 g in Pavon 76 at 40 DAA and 2.14 g in Sonora at 36 days after anthesis.

Under post anthesis heat stress condition, maximum dry matter accumulation in ear and days required to attain maximum dry weight were reduced in all wheat genotypes. The reduction in maximum ear dry weight in heat tolerant, Kanchan (6.12%), Fang 60 (2.32%), BAW 1059 (1.10%) and BL 1022 (9.52%) were lower than that in heat sensitive, Pavon 76 (22.62%) and Sonora (14.0%). Again the in duration required to attain maximum ear dry weight was 4 to 8 days in HT genotypes whereas it was 12 days in Pavon 76 and 16 days in Sonora (Table 5.2).

Kernel dry matter accumulation

Kernel dry matter accumulation followed a typical sigmoid pattern in four heat tolerant (i.e. Kanchan, Fang 60, BAW 1059 & BL 1022) and two heat sensitive (i.e. Pavon 76 and Sonora) wheat genotypes both under normal and heat stress conditions (Fig. 5.8). Under normal growing condition, the kernel dry weight in heat tolerant Fang 60 was found to be increased up to 41.8 mg/kernel at 40 days and declined thereafter slowly. In other three HT genotypes (Kanchan, BAW 1059 & BL 1022), the

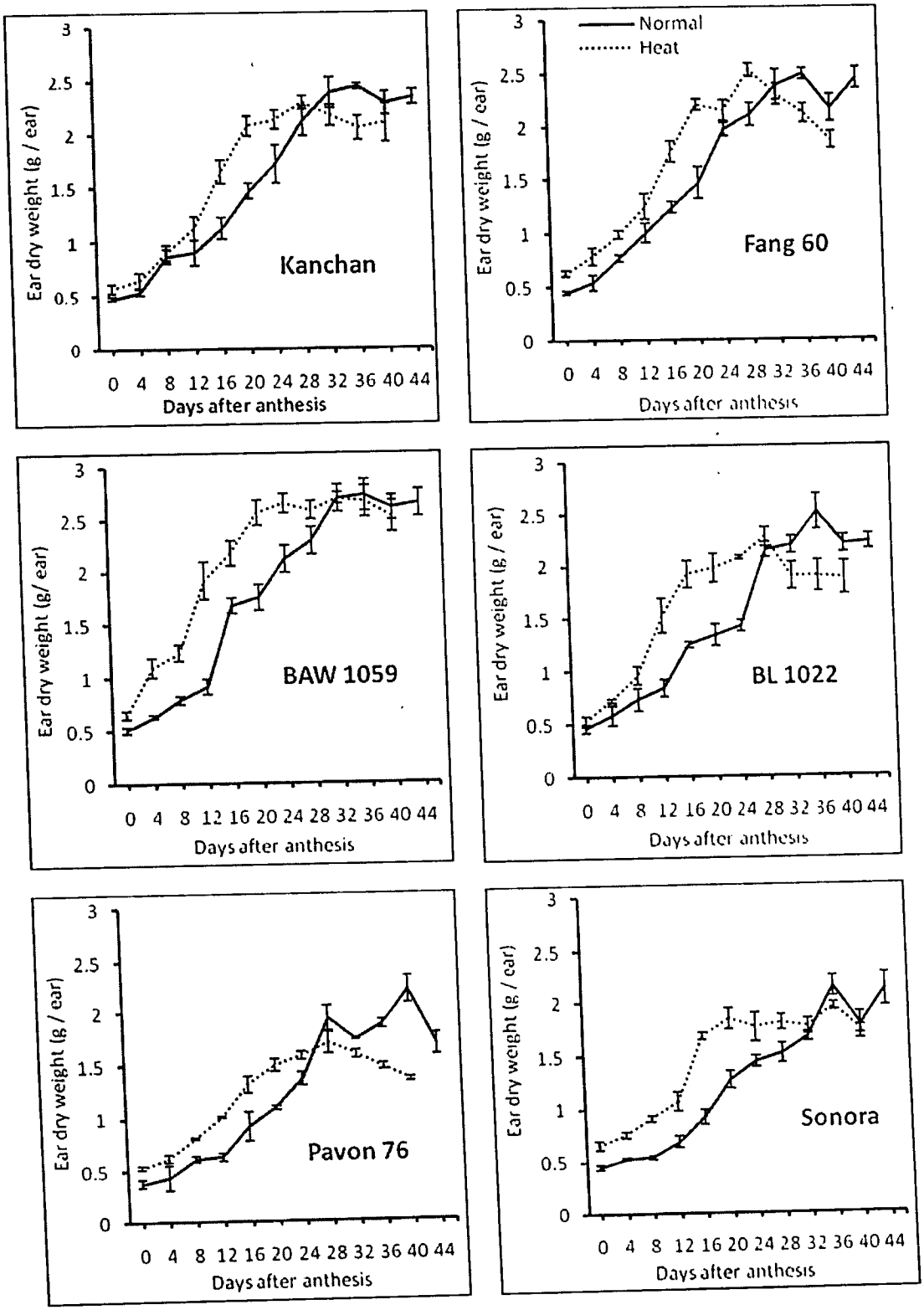


Fig. 5.7. Ear dry weight (mean ± SE) of different wheat genotypes at different days after anthesis as influenced by normal and heat stressed condition.

dry weight of kernel was found to be increased up to 52.64, 51.99 and 50.92 mg/kernel, respectively at 36 days after anthesis. The HS wheat genotypes, Pavon 76 (42.98 mg/kernel) and Sonora (29.72 mg/kernel) also attained their highest kernel dry weight at 36 day after anthesis under normal condition. Due to post anthesis heat stress, maximum kernel dry weight and duration required to attain that kernel dry weight were reduced in all wheat genotypes. The reduction in maximum kernel dry weight due to heat stress was 9.88 to 18.6% in heat tolerant genotypes and it was 11.10 to 27.66% in HS genotypes. The reduction in duration required to attain the maximum kernel dry weight was 4 days in HT genotypes whereas it was 12 days in HS wheat genotypes (Table 5.2). The declining tendency in kernel dry weight after attaining the peak could be due to respiratory loss of kernel after physiological maturity. Sigmoid pattern of kernel dry matter accumulation was also found in wheat by Chanda *et al.* (1999); Karim *et al.* (1999); Sikder *et al.* (1999) and Hasan and Ahmed (2005). Bhatta *et al.* (1994) and Hasan and Ahmed (2005) also found high temperature stress at grain filling period resulted in reduced grain size and grain filling period but reductions were lower in heat tolerant genotypes than those in sensitive genotypes. Wiegand and Cuellar (1981), Bruckner and Frutiberg (1987), Jhala and Jadon (1989), Sikder *et al.* (1999) and Sarker *et al.* (2001) made similar statement.

Kernel growth rate

The absolute kernel growth rate and duration of rapid growth rate (more than 1 mg/day/kernel) varied among wheat genotypes and between growing conditions (Fig. 5.9). Under normal growing condition, all genotypes maintained a longer duration of rapid growth rate, apparently 20-28 days. Due to post anthesis heat stress, this duration was reduced in all genotypes. But the heat tolerant genotypes

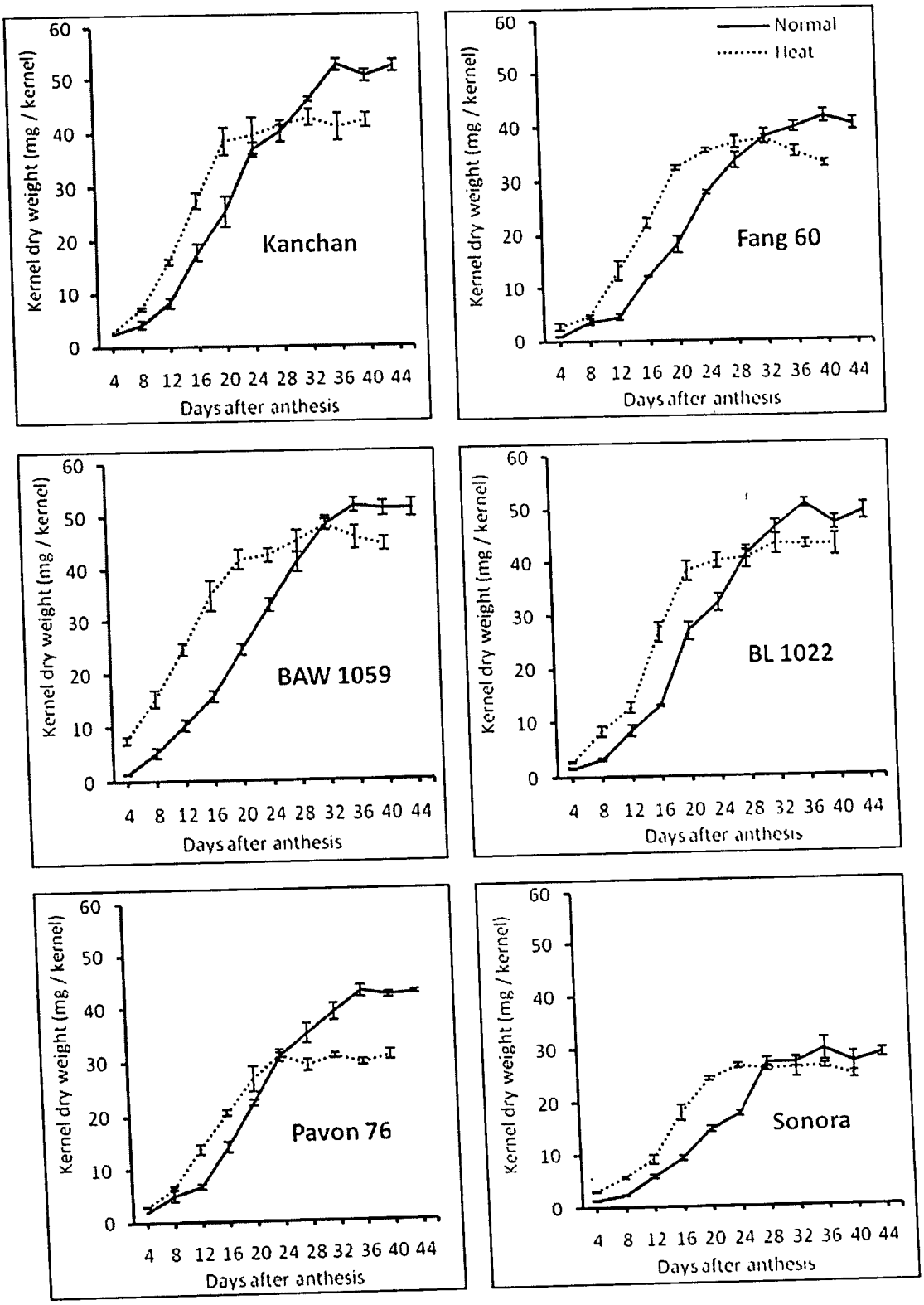


Fig. 5.8. Kernel dry weight of different wheat genotypes at different days after anthesis as influenced by normal and heat stressed condition.

(Kanchan, Fang 60, BAW 1059 & BL 1022) possessed a duration of rapid growth rate of about 16 days whereas the heat sensitive genotype, Pavon 76 maintained that growth rate for 14 days and it was only 10 days for another heat sensitive Sonora. These results agreed with those of Hasan and Ahmed (2005). They also found that duration of rapid growth rate was reduced due to heat stress but reduction was lower in tolerant genotypes than heat sensitive genotypes.

The kernel growth rate data indicate that soon after attaining the highest rate, the plant entered a stage of internal starvation which caused initiation of declining growth rate. At this stage, the grain growth depends largely on its store food materials mobilized from stem. Under normal condition, all genotypes delayed to initiate the phase of internal starvation and as a result, had longer duration of growth rate than heat stress condition. At post anthesis heat stress condition, the HT genotypes initiated starvation phase 4 days earlier. But in both HS genotypes (Pavon 76 and Sonora), internal starvation phase was initiated 12 days earlier than normal condition. Because of their earliest initiation of internal starvation phase HS genotypes had minimum rapid growth duration due to heat stress.

Kernel soluble sugar content

Soluble sugar content in kernel of all wheat genotypes was estimated collecting the samples at 4 days interval from 12days after anthesis to maturity both under normal and heat stress condition and the results expressed as mg/g kernel dry weight were presented in figure 5.10. Under normal growing condition, total soluble sugar in kernel was higher during the early reproductive stage, declined towards maturity with increasing demand for starch synthesis in both HT and HS genotypes. The pattern of soluble

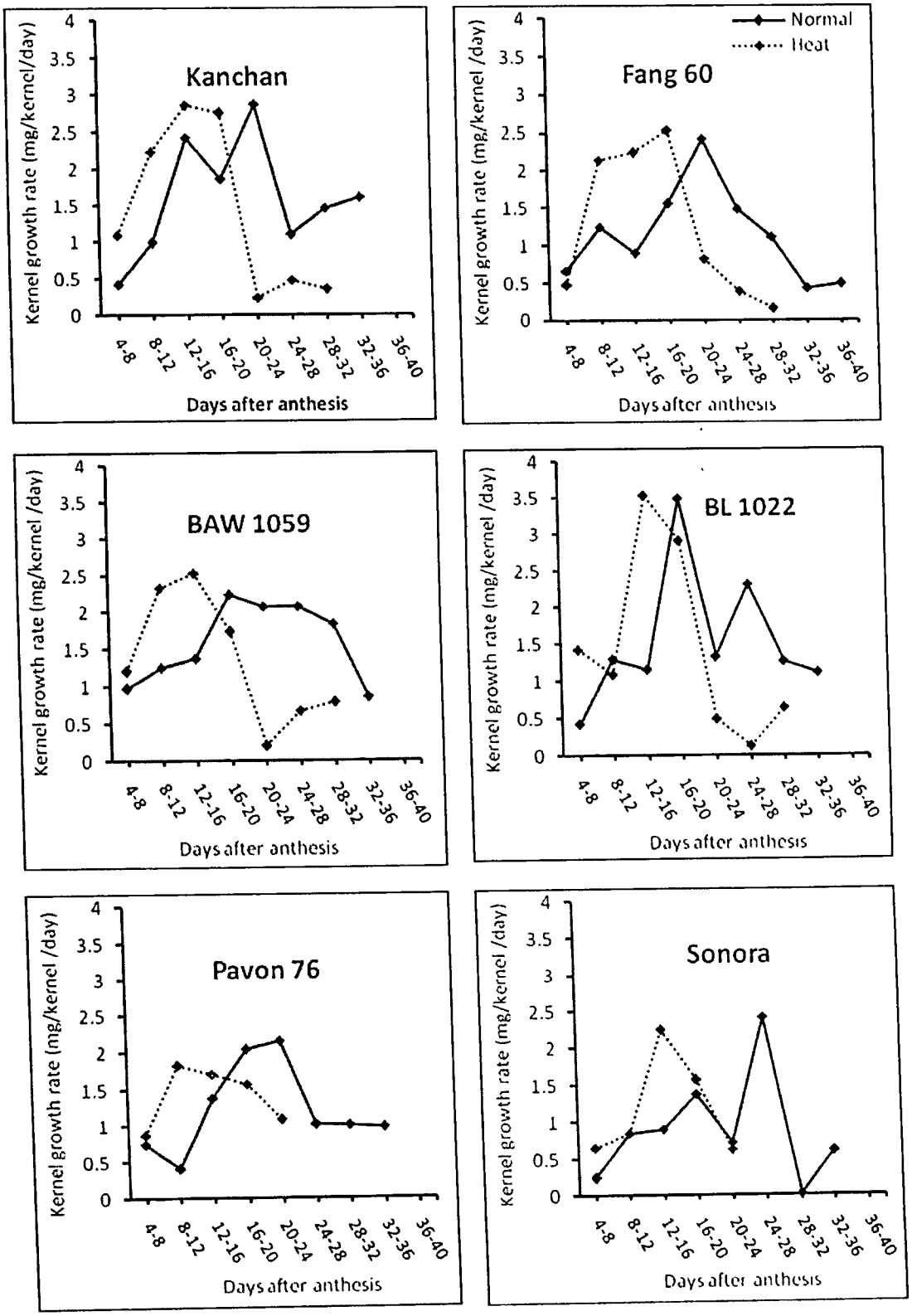


Fig. 5.9. Kernel growth rate of different wheat genotypes at different days after anthesis as influenced by normal and heat stressed condition.

Table 5.2. Leaf longevity, ear and kernel growth duration of different wheat genotypes as influenced by normal and heat stress condition

Genotypes	Growing condition	Leaf longevity (Days)		Ear growth duration (Days)		Kernel growth duration (Days)	
		Actual	Differ.	Actual	Differ.	Actual	Differ.
Kanchan	Normal	30	-	36	-	36	-
	Heat	22	8	28	8	32	4
Fang 60	Normal	32	-	36	-	40	-
	Heat	24	8	28	8	32	8
BAW 1059	Normal	28	-	36	-	36	-
	Heat	20	8	32	4	32	4
BL 1022	Normal	28	-	36	-	36	-
	Heat	20	8	28	8	32	4
Pavon 76	Normal	26	-	40	-	36	-
	Heat	20	4	28	12	24	12
Sonora	Normal	28	-	36	-	36	-
	Heat	16	12	20	16	24	12

sugar levels in kernel revealed that the availability of sugar might become the limiting factor for kernel development in all genotypes under normal condition.

Under late planting heat stress condition, the soluble sugar content in kernel of heat tolerant, Kanchan and Fang 60, was lower (near 40mg/g DW) up to 28 DAA compared to normal condition, thereafter the level of kernel sugar increased than the level existed at normal condition. The results revealed that the availability of sugar was not the limiting factor for kernel development in Kanchan and Fang 60 under heat stress condition. Although the other two HT genotypes, BAW 1059 and BL 1022 maintained a lower soluble sugar content throughout the whole kernel developing period they showed more or less similar trend as shown under normal condition. This result indicated that the availability of soluble sugar might become the limiting factor during the latter kernel growing period. On the other hand, the HS genotypes (Pavon 76 and Sonora) maintained a lower soluble sugar level during the early reproductive growth stage thereafter the level of sugar increased than the level existed under normal condition. 10 to 12 days after attaining physiological maturity of kernel the level of sugar decreased sharply. The early declination of kernel dry matter accumulation and rapid kernel growth rate altogether resulted in rapid increase in kernel soluble sugar in HS genotypes under stress condition. This happened apparently due to limited conversion of sugar to starch rather than limited supply of sugar in kernel. But this limitation (sink activity) appeared 4 to 8 days earlier in HS genotypes compared to HT, Kanchan and Fang 60. Hasan and Ahmed (2005) also found heat stress induced sink limitation in heat sensitive, Sonora.

Heat stress induced sink limitation for kernel development might be due to the limited enzymatic function involving sugar to starch synthesis. At elevated temperature, MacLeod and Dufus (1988), Danyer *et al* (1994) and Jenner (1994) found reduced

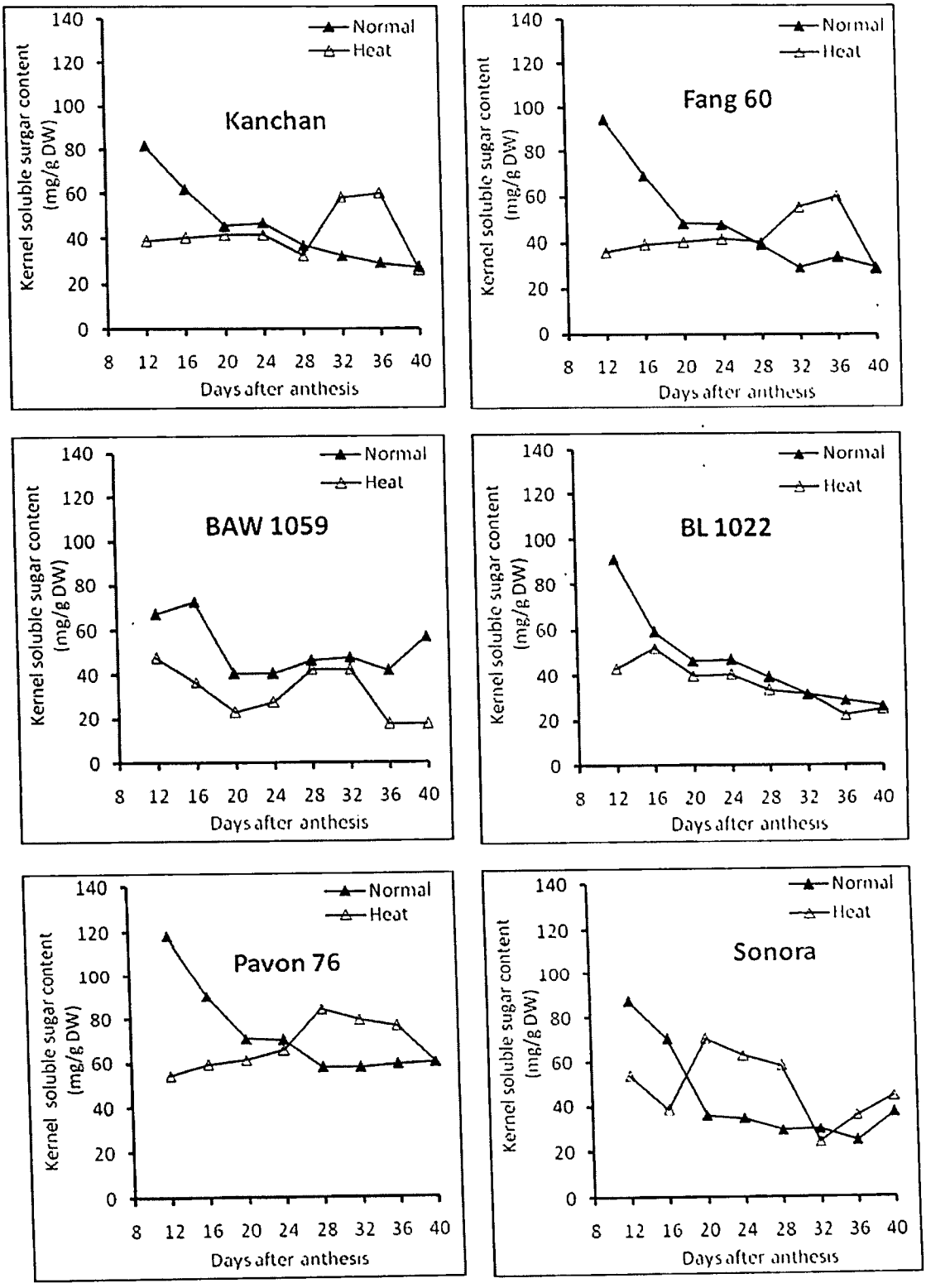


Fig. 5.10. Kernel soluble sugar content of different wheat genotypes at different days after anthesis as influenced by normal and heat stressed condition.

activity of starch synthase in wheat. Keeling *et al* (1994) also reported soluble starch synthase (SSS) as the major site of control of flux through the pathway of starch synthesis in developing wheat grain and temperatures above 25°C adversely affect flux and limit yield.

The overall results in kernel sugar level revealed that the heat tolerant, Kanchan & Fang 60 and heat sensitive, Pavon 76 & Sonora were sink limiting for kernel development under heat stress condition but the limitation appeared 4 to 8 days earlier in HS genotypes than the HT genotypes. The other two HT genotypes, BAW 1059 and BL 1022 were not sink limiting for kernel development and their sink remained active longer time under heat stress condition.

Flag leaf soluble sugar content

Soluble sugar content in flag leaves of all wheat genotypes was determined collecting flag leaves at 4 days interval from 12 days after anthesis to maturity both under normal and heat stress conditions. The levels of flag leaf soluble sugar expressed as mg/g dry weight flag leaf were presented in figure 5.11. Under normal growing condition, each of six wheat genotypes maintained more or less equal of soluble sugar in flag leaves throughout their whole reproductive growth period though this level increased to some extent at later reproductive growth phase in Kanchan and BL 1022.

All the HT genotypes (Kanchan, Fang 60, BAW 1059 and BL 1022) maintained more or less similar pattern of soluble sugar level in flag leaves under heat stress condition as maintained under normal growing condition. On the other hand, both of the HS genotypes (Pavon 76 and Sonora) maintained a higher soluble sugar level in flag leaves up to 28 days after anthesis at heat stress comndition compared to normal

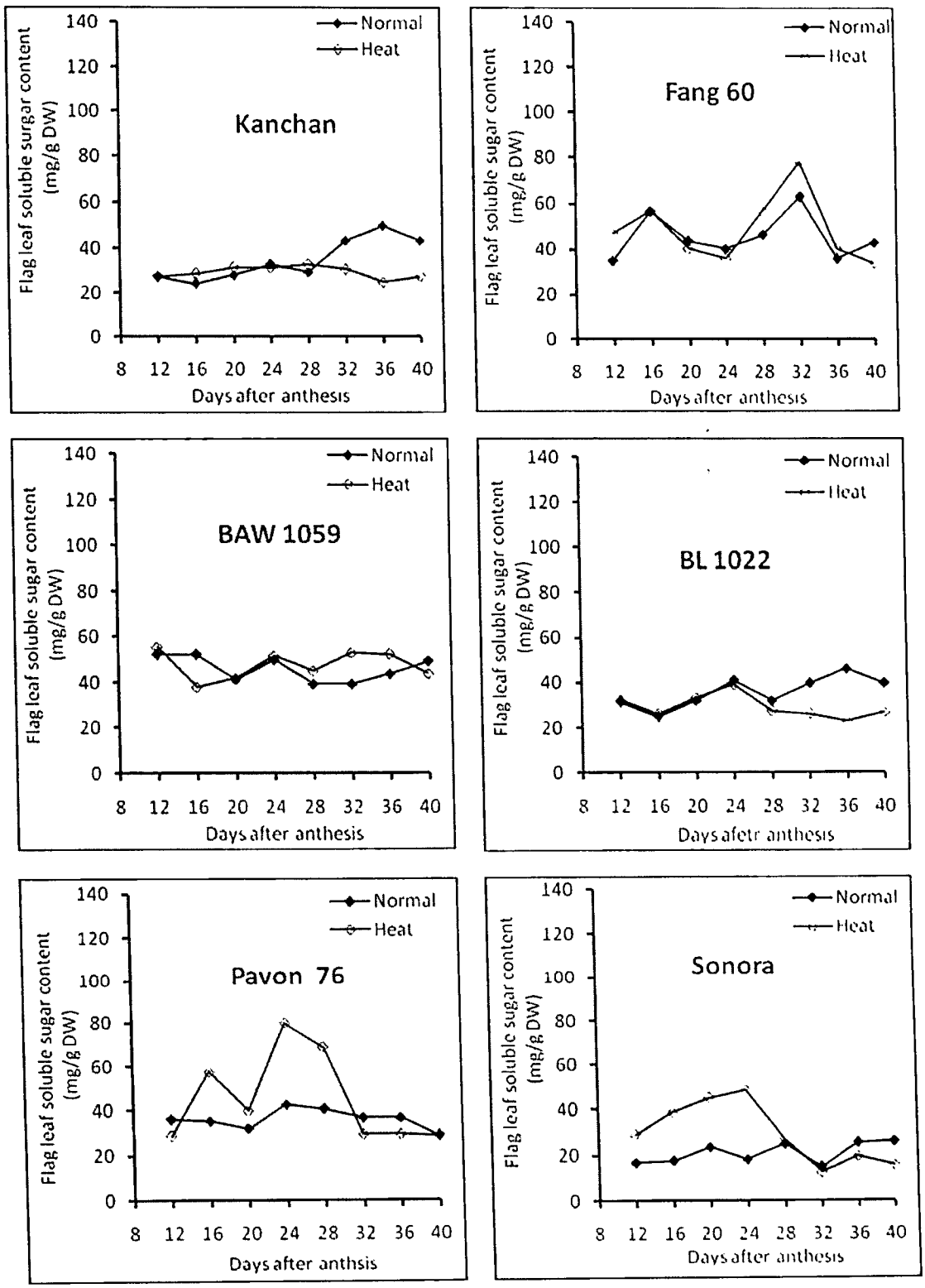


Fig. 5.11. Flag leaf soluble sugar content of different wheat genotypes at different days after anthesis as influenced by normal and heat stressed condition.

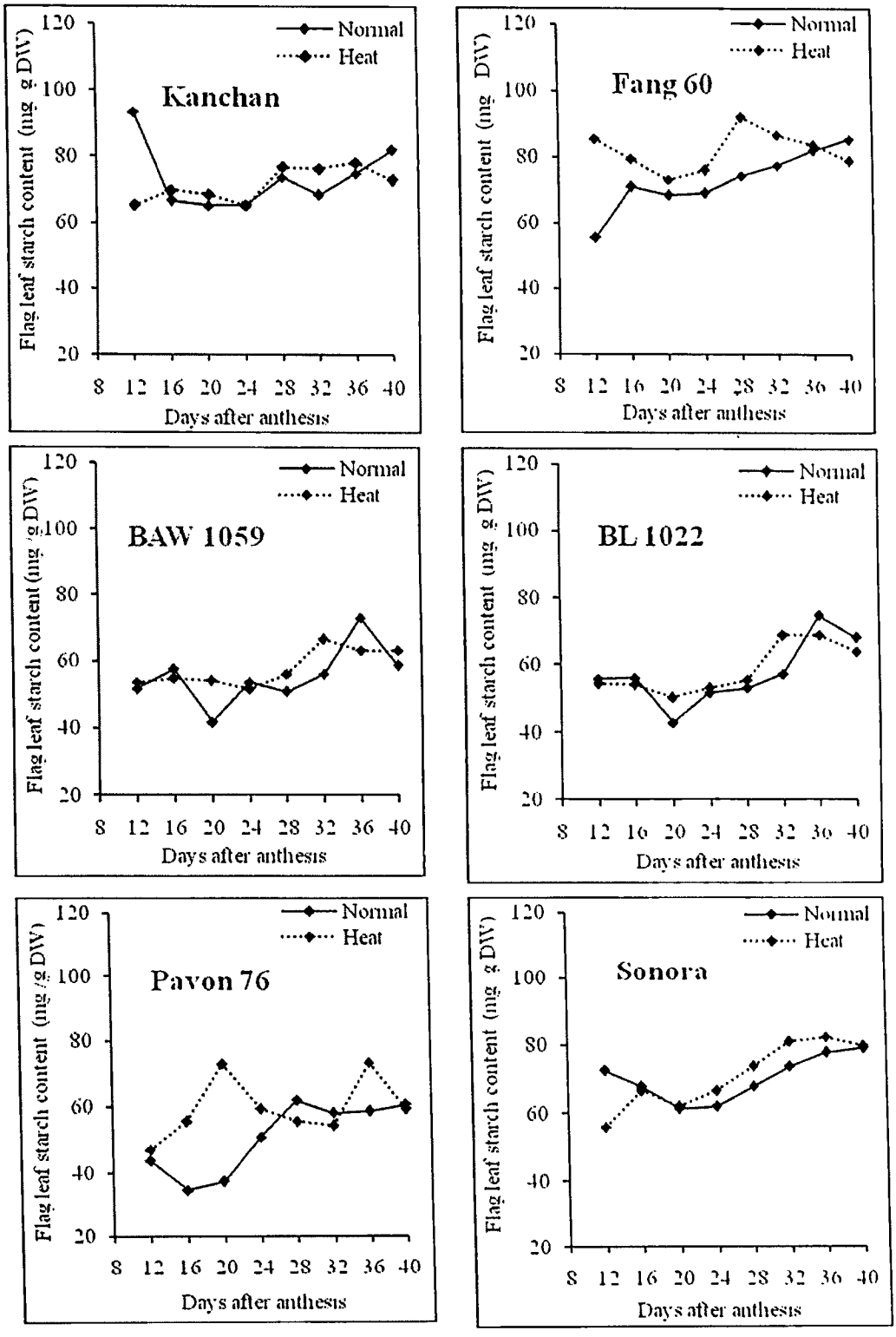
condition. Higher level of soluble sugar before onset of senescence might be due to higher accumulation of photosynthate than it's export.This result also suggested that the sensitivity of HS genotypes to heat stress arises due to sink limitation rather source activity. Excess non- structural carbodyhrate accumulation in flag leaf inhibits leaf photosynthesis via feedback effects (King *et al,* 1967 and Azcon-Bieto, 1983) and also accelerates leaf senescence in wheat (Lazan *et al.*, 1983).

Flag leaf starch content

Starch content in flag leaves of all wheat genotypes was determined collecting flag leaves at 4 days interval from 12 days after anthesis to maturity both under normal and heat stress conditions. The levels of flag leaf starch expressed as mg/g dry weight flag leaf were presented in figure 5.12. Both under normal and heat stress conditions, all wheat genotypes showed lower level of flag leaf starch during rapid grain development stage. Thereafter the flag leaf starch level increased towards the later grain development stage. Lower starch level in flag leaf during the rapid grain development stage might be due to greater sink demand of photosynthates and higher flag leaf starch during the later grain development stage was due to lesser sink demand. Again under heat stress condition, all wheat genotypes maintained higher level of flag leaf starch during the later graing development stage than that under normal growing condition. This might be due to sink sensitivity of wheat to heat stress.

Stem reserve utilization

Different wheat genotypes and growing conditions interacted significantly to influence main stem reserve utilization (Table 5.3). Under normal growing condition,



5.12. Flag leaf starch content of different wheat genotypes at different days after anthesis as influenced by normal and heat stress condition.

main stem reserve utilization was the highest in Kanchan (0.72 g/main stem) which was statistically identical with that in BAW 1059 (0.68 g/main stem). Fang 60 followed the BAW 1059 in utilizing main stem reserve (0.46 g/main stem). Main stem reserve utilization was the lowest in BL 1022 (0.38 g/main stem) which was statistically similar to that in Pavon 76 (0.41g/main stem) and Sonora (0.42 g/main stem).

Due to late planting heat stress, the stem reserve utilization was significantly reduced in two HT (Kanchan and Fang 60) and HS (Pavon 76 and Sonara) genotypes though the reduction was higher (64 to 71%) in HS genotypes compared to that in HT genotypes (33 to 49%). In another two HT genotypes, BAW 1059 and BL 1022, stem reserve utilization was even increased due to heat stress though the increment was not significant. The relative value (in heat stress condition compared to normal condition) indicated that the HT genotypes (0.51 to 1.11) had greater ability to utilize stem reserve compared to HS genotypes (0.29 to 0.36).

Shukla *et al.* (1997) indicated greater stem reserve mobilization in tolerant genotypes to support grain filling during critical stages of dry matter accumulation in the grain compared to heat sensitive genotypes. Blum *et al.* (1994) also concluded that the superior capacity of heat tolerant genotype for grain filling from mobilized stem reserves is a constitutive trait which supports grain filling under heat stress.

Stem reserve utilization in HT, Kanchan & Fang 60, and also in HS, Pavon 76 & Sonora, was reduced due to heat stress. Because in the present study, these four wheat genotypes were found as sink limiting for grain development under heat stress condition. Again two HS genotypes showed greater reduction in stem reserve due to heat stress. This may be due to their sink limitation appeared 4 to 8 days earlier than

Table 5.3. Stem reserve utilization of different wheat genotypes as influenced by normal and heat stress condition

Genotypes	Reserve utilization (g/ main shoot)		
	Normal growing condition	Heat stressed condition	Relative to normal
Kanchan	0.72 a	0.37 c	0.51
Fang 60	0.46 b	0.31 d	0.67
BAW 1059	0.68 a	0.72 a	1.06
BL 1022	0.38 c	0.42 bc	1.11
Pavon 76	0.41bc	0.12 e	0.29
Sonora	0.42 bc	0.15 e	0.36
CV (%)	7.89		-

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

the HT genotypes. Increased utilization of stem reserve due to heat stress was found in other two HT genotypes (BAW 1059 and BL 1022).This may me due to their grain had greater ability to utilize reserve photosynthate as they were not indentified as sink limiting genotypes under heat stress condition.

Grain dry weight per main stem ear

Significant variation was found in grain dry weight per main stem ear by the interaction effect of growing conditions and wheat genotypes (Fig. 5.13). Under normal growing condition, BAW 1059 obtained the highest grain dry weight per main stem ear (2.22 g/ear) which was statistically at par with that obtained from BL 1022 (2.06 g/ear). Sonora produced significantly the lowest grain dry weight per main stem ear (1.69 g/ear) which was followed by Pavon 76 (1.83 g/ear) though they did not differ significantly. Whereas the grain dry weight produced on the main stem ear of Kanchan (1.97 g/ear) and Fang 60 (2.04 g/ear) were medium although they did not differ significantly each other. Both of the genotypes differed significantly with all other genotypes under control condition, except Kanchan with Pavon 76 and Fang 60 with BL 1022.

Due to late planting heat stress, the grain dry weight per main stem ear was reduced significantly in all wheat genotypes except in BAW 1059. But different genotypes showed different degree of responses to heat stress. The reduction in grain dry weight per main stem ear was the least in BAW 1059 (5%) which was followed by Fang 60 (7%), Bl 1022 (10%) and Kanchan (12%). Whereas the reduction was the highest in Pavon 76 (36%) this was followed by Sonora (30%). The reduction grain dry weight per main stem ear under heat stress condition might be due to reduced grain size in HT genotypes but in HS genotypes due to combine effect of reduce grain size and reduced grain number/ ear (Table 5.5). Fokar *et al.* (1998b) observed significant

variation among five wheat cultivars in the reduction in grain weight per ear, kennel number and single kennel weight under heat stress. Differences in grain weigh per ear among cultivars were ascribed to the variation in the reduction in both kennel number and kernel weight under heat stress. Shpiler and Blum (1986) observed that the cultivars that sustained the highest yield in hot environments were able to maintain the longest duration of GS_2 and had higher number of grain per spike. High temperature (up to 30/25 °C day/night) after anthesis reduced grain yield of wheat by reducing weight per grain rather than grain number (Wardlaw *et al.*, 1989). Figure 5.14 showed the relative performance of different wheat genotypes in producing grain per main stem ear under heat stress. The relative value indicated that HS genotypes affected more (0.64 to 0.70) than the HT genotypes (0.90 to 0.95) in producing grain per main stem ear under heat stress. The heat tolerance of different wheat genotypes in terms of grain dry weight per main stem ear was found to be significantly correlated ($r = - 0.968^{**}$, $n = 6$) with the heat tolerance based on percent membrane injury (Fig. 5.15).

Grain dry weight per main stem ear is the ultimate sink size of the main stem. In the present study, wheat genotypes, BAW 1059 and BL 1022 were not indentified as sink limiting due to heat stress as a result, showed greater ability to utilize stem reserve for grain development. Longer grain growth duration and greater ability to utilize stem reserve contributed to produce greater sink size under stress condition in these HT genotypes. Other two HT genotypes (Kanchan and Fang 60) were identified as sink limiting but their limitation arisen 28 DAA as a result, showed moderate ability to utilize stem reserve for grain development consequently, produced moderate sink size. The sink of the HS were found as very sensitive to heat stress and they have lesser ability to utilize stem reserve for grain development consequently produced moderate sink size i. e. grain dry weight per main stem ear under stressed condition.

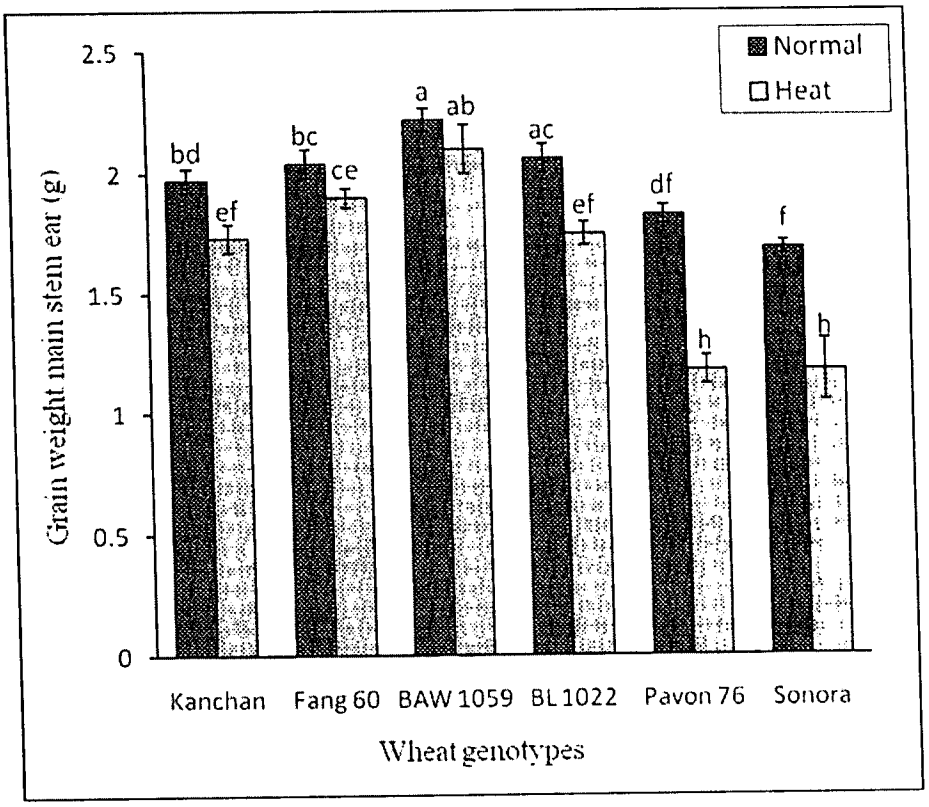


Fig. 5.13. Main stem grain dry weight of different wheat genotypes as influenced by normal and heat stress condition.

Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

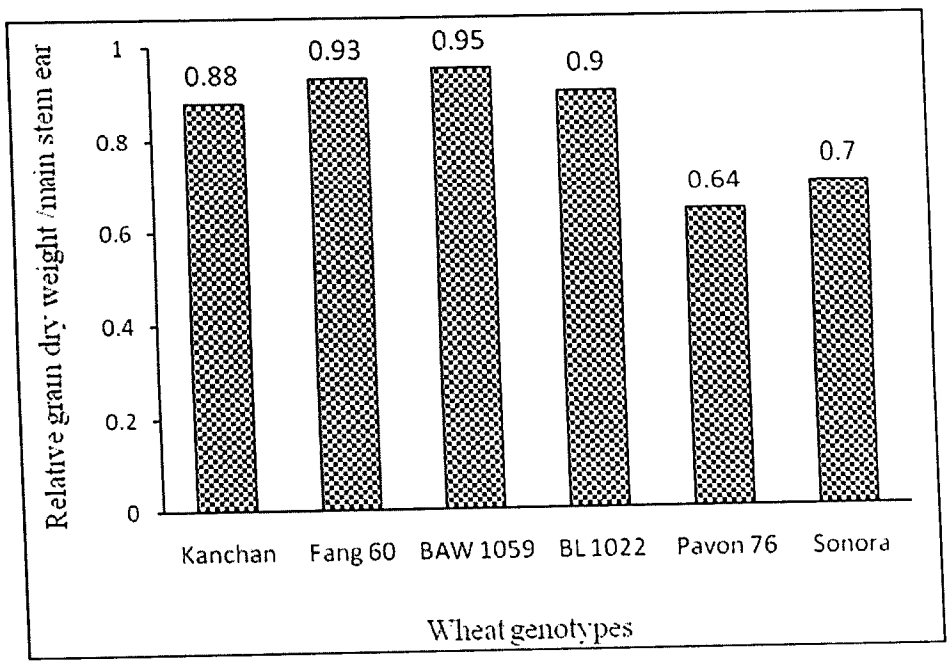


Fig. 5.14. Relative grain dry weight per main stem ear of different wheat genotypes.

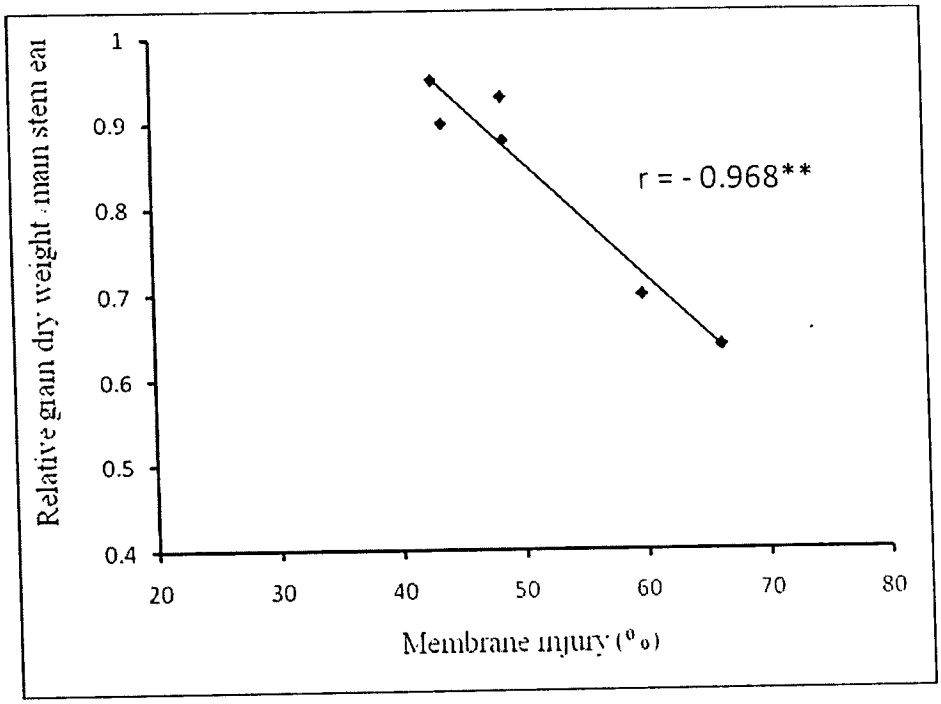


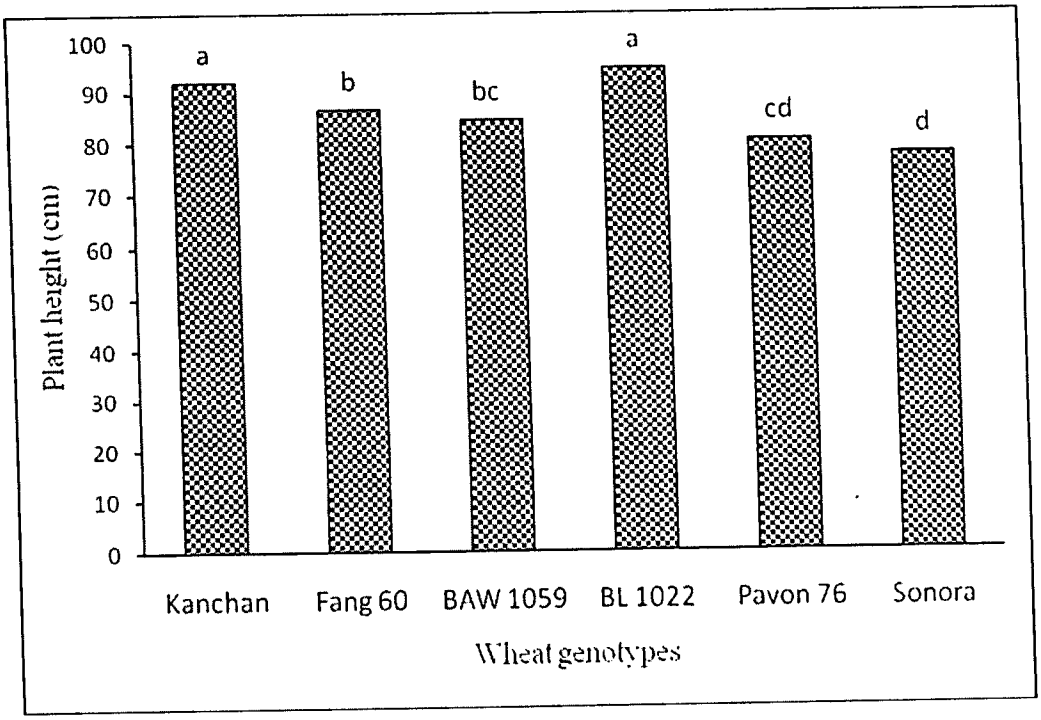
Fig. 5.15. Relationship (r = -0.968**, n = 6) between membrane injury (%) and relative grain dry weight per main stem ear of different wheat genotypes.

Plant height

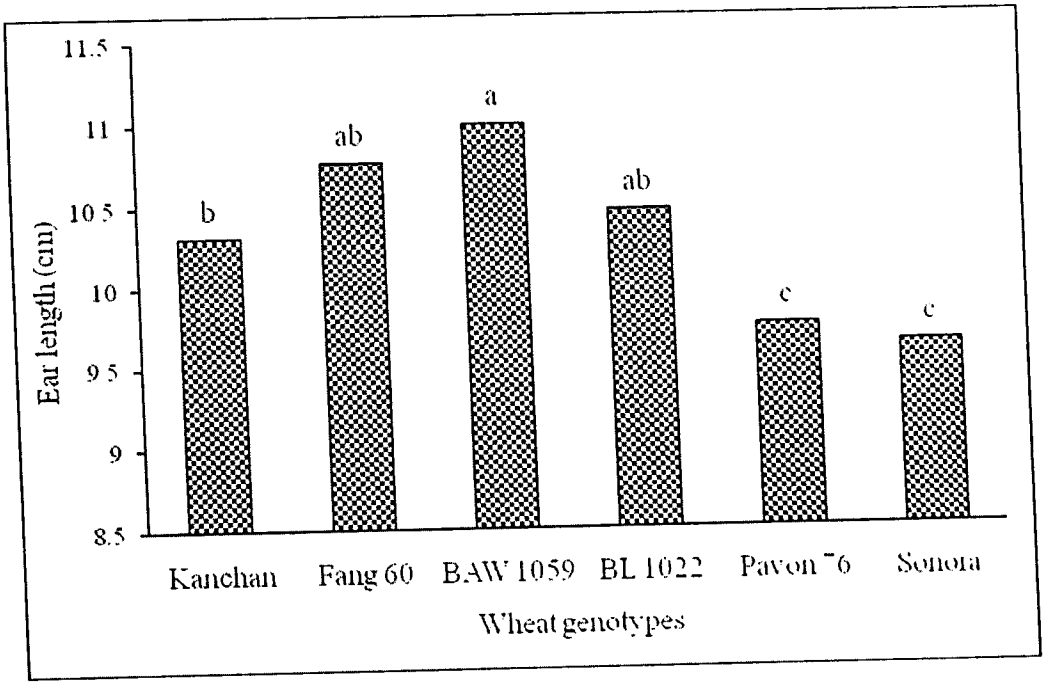
Plant height was significantly influenced by wheat genotypes but the effect of growing condition or the combine effect of growing conditions and wheat genotypes on plant height was insignificant (Fig. 5.16). Irrespective of growing condition, Bl 1022 was the tallest (94.63 cm) genotype which was followed by Kanchan (92.11 cm) but they did not differ significantly in height. Sonora (77.58cm) was the shortest genotype which was followed by Pavon 76 (80.39 cm) but there was no significant difference between them. Whereas Fang 60 (86.52 cm) and BAW 1059 (84.47 cm) were moderate in height. The differences in plant height among the genotypes were due to the genetic variation. Due to heat stress, plant height reduced in all genotypes but the reduction was not significant. With delay planting, the development of plant organs and transfer from source and sink were remarkably affected, which was reflected by overall shortening of plant height (Sial *et al.*, 2005).

Ear length

The combine effect of growing conditions and wheat genotypes did not influence the ear length significantly though it was influenced significantly by both growing conditions and wheat genotypes (Fig. 5.17). Irrespective of growing conditions, BAW 1059 (11.00 cm) produced the longest ear which was statistically equal to that produced by Fang 60 (10.77 cm) and Bl 1022 (10.47 cm). The HS Sonora produced significantly the shortest ear (9.63 cm) which was statistically equal to that produced by Pavon 76 (9.75 cm). The other HT genotype, Kanchan produced a moderate ear in length (10.32 cm). Due to heat stress, the ear length was reduced somewhat in all wheat genotypes except in Fang 60. The ear length of Fang 60 remained unchanged in both their growing conditions.



5.16. Plant height of different wheat genotypes. Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.



5.17. Ear length of different wheat genotypes. Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

Number of ear

The combine effect of growing conditions and wheat genotypes on number of ears per m^2 was significant (Table 5.5). At normal growing condition, Pavon 76 produced the highest number of ears per m^2 (522) which was statistically similar to that of Sonora (512) and Fang 60 (486). BAW 1059 produced significantly the lowest number of ears per m^2 (359). Other two heat tolerant genotypes, Kanchan (423) and BL 1022 (432) produced a moderate number of ears per m^2 though they did not differ statistically but both of them differed statistically from all other genotypes. Due to late planting heat stress condition, ear number per m^2 was reduced significantly in all wheat genotypes though the reduction was insignificant in Kanchan.

The HS genotype showed the lower relative value (0.56 to 0.70) in number of ears per m^2 compared to that in HT genotypes such as , Kanchan (0.97) Fang 60 (0.76), BAW 1059 (0.75) and BL 1022 (0.87), indicating that late planning heat stress affects the HS wheat genotypes more than the HT genotypes. Sail *et al.* (2005) and Islam *et al* (1993) also found reduced number of ears/m^2 in heat stressed environment.

Number of grains per ear

Grain number per ear was influenced significantly by the interaction effect of growing conditions and wheat genotypes (Table 5.5). Under normal growing condition, Fang 60 had the highest number of grain per ear (47.4) which was statistically similar to that of Sonora (47.3). Kanchan had the lowest grains per ear (29.6) whereas, in case of BL 1022, it was the second lowest (34.45). Other two genotypes (BAW 1059 and Pavon 76) were moderate in producing grains per ear and the difference between them was not significant. On the other hand, at post anthesis heat stress condition, this number was reduced in HS genotypes (Sonora and Pavon 76) though the reduction

was not significant. But in HT genotypes (Kanchan, Fang 60, BAW 1059 and BL 1022), it was even increased and the increment was significant except Kanchan.

Significant variation among different wheat genotypes in the reduction in grain number/ear under heat stress was found by Fokar *et al.* (1998a), Sial *et al.*, 2005 and Wollenweber *et al.* (2003). Shpiler and Blum (1986) observed that the cultivars that sustained the highest yield in hot environments were able to maintain the longest duration of GS_2 and had higher number of grain per spike.

Grain size

Individual grain size was differed significantly due to the combine effect of growing conditions and wheat genotypes (Table 5.5). Under normal growing condition, maximum grain size was recorded in Kanchan (48.27 mg/grain) which was statistically equal with that of BL 1022 (47.82 mg/grain) and BAW 1059 (47.01 mg/grain). Sonora obtained the lowest grain size (30.40 mg/ear) which followed the kernel size of Pavon 76 (32.41 mg/grain). Fang 60 produced the medium grain size (34.72 mg/grain) which differed significantly from all other wheat genotypes. These deviations in grain size among the wheat genotypes were due to their genetic variation. At post anthesis heat stress condition, grain size reduced significantly in all wheat genotypes. But the magnitude of reduction was different in different genotypes. In this condition, the HS genotypes attained a relative kernel size of 0.78 to 0.80 but in the HT genotypes it varied from 0.75 to 0.96. Reduced grain size under heat stress condition might be due to the reduction in rapid kernel growth duration. Significant variation in reduction in grain size under heat stress among different wheat genotypes was observed by Fokar *et al.* (1998a), Islam *et al.* (1993), Sial *et al.*, 2005 and Wollenweber *et al.* (2003).

Table 5.4. Yield characters of different wheat genotypes as influenced by normal and heat stress conditions

Genotypes	Growing condition	Ear/m^2		No. of grain/ear		Grain size (mg)	
		Actual	Relative	Actual	Relative	Actual	Relative
Kanchan	Normal	423 b	-	29.6 f	-	48.27 a	-
	Heat	411bc	0.97	33.2 ef	1.12	36.40 b	0.75
Fang 60	Normal	486 a	-	47.4 b	-	34.72 c	-
	Heat	368 d	0.76	51.5 a	1.09	26.52 f	0.76
BAW 1059	Normal	359 d	-	38.3 cd	-	47.01 a	-
	Heat	271 e	0.75	45.3 b	1.18	37.36 b	0.79
BL 1022	Normal	432 b	-	34.4 de	-	47.82 a	-
	Heat	374cd	0.87	39.2 c	1.14	33.73cd	0.96
Pavon 76	Normal	522 a	-	38.8 c	-	32.41 d	-
	Heat	366 d	0.70	36.5 ce	0.94	25.36fg	0.78
Sonora	Normal	512 a	-	47.3 b	-	30.40 e	-
	Heat	286 e	0.56	47.0 b	0.99	24.29 g	0.80
CV(%)		5.98	-	5.74	-	2.76	-

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

Grain yield

Growing conditions and wheat genotypes interacted significantly to govern the grain yield (Table 5.6). Under normal growing condition, Fang 60 attained the highest grain yield (580.3 g/m^2) which was followed by BAW 1059 (561.7 g/m^2) but they did not differ significantly. Kanchan (509.6 g/m^2), BL 1022 (468.7 g/m^2), Pavon 76 (476.1 g/m^2) and Sonora (479.1 g/m^2) gave significantly the lower grain yield compared to Fang 60 and BAW 1059. These for wheat genotypes did not differ significantly among them though Pavon 76 gave the lowest yield. Post anthesis heat stress condition decreased the grain yield significantly in all wheat genotypes but the magnitude of reduction was not equal in different genotypes. The reduction in grain yield was greater in HS genotypes (45 to 55%) than those in HT genotypes (27 to 38%). The relative yield to normal also indicated that the HS genotypes (0.45 to 0.55) affected more in producing grain yield than the HT genotypes (0.62 to 0.73).

Reduced number of ears per m^2 and reduced grain size were the major responsible factors for reducing the grain yield under heat stress condition in the present experiment. Reduction in number of grains per ear greatly contributed to reduce grain yield further in HS genotypes. But in HT genotypes, number of grains per ear remained stable or even increased to support the tolerance in terms of grain yield. Results from other studies showed that late planting heat stress caused lower grain yield in wheat compared to optimum sowing (Islam *et al.*, 1993; Bhatta *et al.*, 1994, Rasal *et al.*, 2006, Poonam *et al.*, 2006, Sial *et al.*, 2005, Wollenweber *et al.*, 2003). Significant variation due to heat stress in different wheat genotypes was also found by Rasal *et al.* (2006), Hasan and Ahmed (2005). Al-Khatib and Paulsen, (1990) concluded the high relative grain yield which was the results of stable and/or long

duration of phosynthetic activity at heat stress condition as a selection criterion for heat tolerance of wheat genotypes.

Above ground biological yield

Above ground biological yield of wheat was significantly influenced by the interaction effect of growing conditions and genotypes (Table 5.6). Under normal growing condition, Fang 60 gave the highest above ground biological yield (1270.2 g/m^2) that was statistically at par with that obtained from Kanchan (1226.8 g/m^2), BAW 1059 (1196.8 g/m^2) and BL 1022 (1208.7 g/m^2). Sonora gave significantly the lowest biological yield (1050.2 g/m^2) which was followed by Pavon 76 (1071.1 g/m^2) and the difference in above ground biological yield between Sonora and Pavon 76 was not significant. Under heat stress condition, above ground biological yield decreased significantly in all wheat genotypes but the degree of reduction was not similar in all genotypes. The HT genotypes, Kanchan, Fang 60, BAW 1059 and BL 1022 showed greater reduction (16 to 30%) in above ground biological yield than the HS genotypes (35 to 39%). The relative above ground biological yield to normal showed that the HS genotypes affected more (0.61 to 0.65) than the HT genotypes (0.70 to 0.84). The reduced above ground biological yield in wheat under heat stress was due to the reduced grain and straw yield. Bhatta *et al.* (1994), Hasan and Uddin (2005), Wollenweber *et al.* (2003) also reported reduced biological yield under high temperature.

Heat susceptibility index

Heat susceptibility index based on grain yield varied in different wheat genotypes (Fig. 5.18). According to the susceptibility index, Kanchan (0.83), Fang 60 (0.93) BAW 1059 (0.98) and BL 1022 (0.70) were found as HT genotypes among them BL 1022 was the most tolerant. On the other hand, Pavon 76 (1.42) and Sonora (1.17)

Table 5.5. Grain yield and above ground biological yield of different wheat genotypes as influenced by normal and heat stress conditions

Genotypes	Growing condition	Grain yield (g/m^2)		Above ground biological yield (g/m^2)	
		Actual	Relative	Actual	Relative
Kanchan	Normal	509.6 b	-	1226.8 a	-
	Heat	347.1 c	0.68	1005.3 b	0.82
Fang 60	Normal	580.3 a	-	1270.2 a	-
	Heat	373.2 c	0.64	919.7 c	0.72
BAW 1059	Normal	561.7 a	-	1196.8 a	-
	Heat	351.6 c	0.62	837.3 d	0.70
BL 1022	Normal	468.7 b	-	1208.7 a	-
	Heat	342.7 c	073	1012.3 b	0.84
Pavon 76	Normal	476.1 b	-	1071.1 b	-
	Heat	218.0 d	0.45	692.5 e	0.65
Sonora	Normal	479.1 b	-	1050.2 b	-
	Heat	262.1 d	0.55	639.7 e	0.61
CV (%)		7.97	-	4.37	-

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

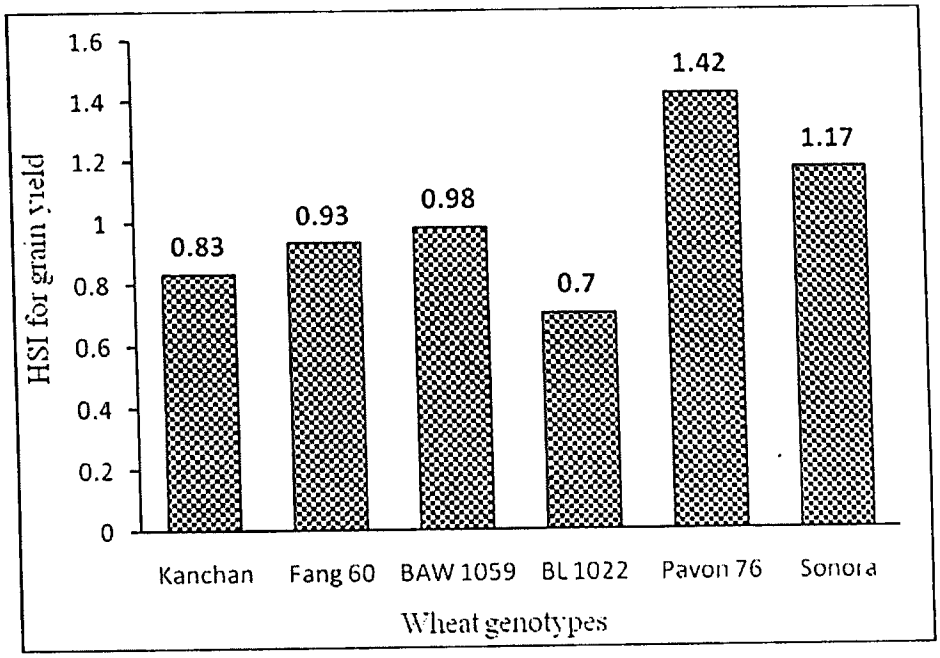


Fig. 5.18. Heat susceptibility index (HSI) for grain yield of different wheat genotypes (Higher HIS indicates greater susceptibility).

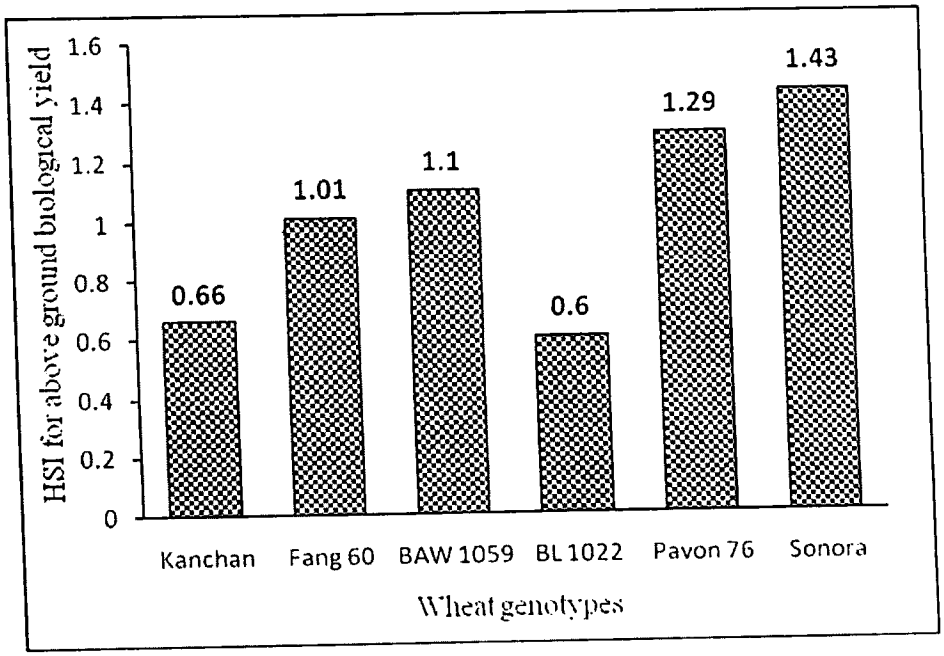


Fig. 5.19. Heat susceptibility index (HIS) for above ground biological yield of different wheat genotypes (Higher HIS indicates greater susceptibility).

were identified as heat susceptible genotypes between them Pavon 76 was more susceptible genotype. Heat susceptibility index based on above ground biological yield also varied in different wheat genotypes (Fig. 5.19). Based on the susceptibility index, Kanchan (0.66) and and BL 1022 (0.60) were found as heat tolerant genotypes. On the other hand, Fang 60 (1.01), BAW 1059 (1.10), Pavon 76 (1.29) and Sonora (1.43) were identified as heat susceptible genotypes. Among them Pavon 76 and Sonora showed greater susceptibility than Fang 60 and BAW 1059. Hasan and Ahmed (2005) found that the heat tolerant genotypes showed lower susceptibility index (0.346 to 0.962) than the heat sensitive genotype (1.721). Heat stress reduced the grain yield but low heat susceptibility indexes for grain yield were found in heat tolerant genotypes (Rasal *et al.*, 2006).

The results of the present study indicate that distinct changes in kernel proline level between HT and HS wheat genotypes was found at early reproductive development and in flag leaf, it was found at later reproductive growth due to heat stress. Flag leaf longevity reduced in all wheat genotypes due to late planting heat stress but the reduction was inconsistent between HT and HS wheat genotypes. The heat tolerant wheat genotypes showed greater ear and grain growth duration, longer duration rapid grain growth rate, higher stem reserve utilization and consequently maintained lesser reduction in grin dry weight per ear and grain yield under heat stress condition than those showed by heat sensitive wheat genotypes. The HT genotypes, Kanchan & Fang 60 and the HS genotypes, Pavon 76 & Sonora were sink limiting for kernel development under heat stress condition but the limitation appeared 4 to 8 days earlier in HS genotypes than the HT genotypes. The other two HT genotypes, BAW 1059 and BL 1022 were not sink limiting for kernel development and have longer sink activity under heat stress condition.

Experiment 4. SUSTAINING WHEAT YIELD WITH EXTRA NITROGEN MANAGEMENT UNDER LATE PLANTING HEAT STRESSED ENVIRONMENT

INTRODUCTION

In Bangladesh most of the wheat is cultivated at late sowing condition after harvesting transplanted aman rice. High temperature during post anthesis phase is a common feature for late planting wheat. A further dimension is added to this problem by the projected rise in global mean temperature and the frequency of periods of post anthesis high temperature which may further increase the pressure of heat stress in wheat production (Conroy *et al.* 1994). An early senescence of photosynthetic system (Al-Khatib and Paulson, 1990) and reduction in grain filling period as well as the rate of grain filling (Sofield, 1977, Hasan and Ahmed, 2005) under post anthesis high temperature stress have been indicated as casual factors for a yield penalty of 10-15% per ear (Wardlaw and Wrigley, 1994).

Wheat grain filling depends on two major sources of carbon: current photosynthesis in leaves and ears, and the mobilization of stored reserve from the stem in to the growing grain. Stored assimilates are an important source for grain filling in wheat and up to 0.78g of grain may be produced from 1g of assimilates stored in wheat stover (Kiniry, 1993). When the current photosynthetic source is inhibited, grain filling becomes more dependent on mobilized stem reserves (Bindinger *et al.*, 1977; Blum, 1988).

A non-limiting supply of resources is required for optimal plant growth and at above optimum temperature the demand of resources is higher due to increased rate of metabolism, development and evapo-transpiration (Rawson, 1988). When growth resources are limited by heat stress, the size of plant organs such as leaves, tillers and spikes is reduced (Fischer, 1985). Under high temperature conditions, volatilization of

N fertilizers such as NH_3 is more likely and further decreases wheat yield (Tran-Thuc-Son *et al.*, 1995). Nitrogen contents in leaves reduces by 10% when wheat plants experience heat stress at the double ridge stage and by 25% at anthesis stage (Woolenweber *et al.*, 2003). Additional amount N fertilizer may be needed to sustain late planting yield reduction due to heat stress. Application of extra nitrogen or splitting of N fertilizer after anthesis may provide some benefits either by lengthening the leaf activity and/or by increasing stem reserve which might be utilized later for grain development. The present study was therefore conducted to evaluate the influence of application of extra nitrogen and/or splitting of nitrogen fertilizer after anthesis to sustain wheat yield due to late planting heat stress.

MATERIALS AND METHODS

Location and duration

The experiment was carried out at the plot (No.14) of the research farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during November, 2007 to Octobar, 2008 on an upland soil. It is located at the center of Madhupur Tract (24°05' N latitude and 90°25'E longitude) at an elevation of 8.4 m above the sea level.

Soil and climate

The soil of BSMRAU farm belongs to Sanla series of Shallow Red-Brown Terrace soil type with silty clay in surface and silty clay loam in sub-surface region. As per USDA soil classification, the experimental soil was under Ochrept sub-order of Inceptisol order. The information of basic physical and chemical properties of the soil are presented in Appendices 1and 2. The experimental site is situated in the sub-tropical region characterized by heavy rainfall during the months from May to September and scantly rainfall in the rest of the year. Meteorological data on rainfall, temperature, relative humidity and evaporation from December 2007 to April 2008 obtained from meteorological station of Plant Physiology Division, BRRI, Gazipur are presented in Appendix 4.

Experimental design and treatment

The experiment was conducted in two factors randomized complete block design with three replications. The unit plot size was 3 m x 2 m having a plot to plot and block to block distance of 0.5 m and 1.0 m, respectively.

dose. In case of T_1, T_2 and T_3, recommended amount of N fertilizer and in case of T_4 and T_5, recommended + extra 50% N fertilizer was applied as cited in factor B. Irrigation were given to avoid the drought stress when necessary.

Sowing

Seeds of four wheat genotypes (Kanchan, Fang 60, BAW 1059 and Sonora) were sown on November 30 and December 30, 2007 in rows of 20 cm apart, at the rate of 120 kg/ha. November 30 sowing was regarded as normal and December 30 sowing as post anthesis heat stress growing conditions. After sowing slight irrigation with overhead sprinkler was given for uniform germination.

Intercultural operations

After sowing care was taken against birds up to 15 days. The crop was kept weed free and for controlling diseases, Tilt 250 EC was sprayed regularly at 15 days interval after 30 days of sowing.

SPAD value

Five main shoots were selected from each plot at the time of anthesis and tagged. SPAD value was taken from middle portion of the flag leaf of the tagged main shoot at 2 days intervals from anthesis to senescence using Minolta chlorophyll meter (Model: SPAD-502, Minolta Co. Ltd., Japan). Measurements at each experimental plot consisted average of five readings.

Ear growth rate and stem reserve utilization

At anthesis 60 main shoots were tagged from each plot. Four tagged main shoots were harvested at every 4th day beginning from anthesis to quantify ear growth and stem reserve utilization. The harvesting of main shoots from all experimental units was continued up to 44 days after anthesis (DAA) for normal growing condition

(November 30 sowing) and 40 DAA for post anthesis heat stress conditions (December 30 sowing). The harvested main shoots were separated in to ears and stem with leaf sheath. The ears and stem were kept in oven at 70^0C for 72 hours. After oven drying ears and stems were weighted with an analytical balance (AND Electronic Balance Model ER 180A A & D Company Limited, Tokyo, Japan).

Stem reserve utilization (g/ main shoot) was calculated using the formula from the model shown in experiment 3 using the following formula-.

Stem reserve utilization (g / main shoot) =

Maximum stem dry weight – Stem dry weight at physiological maturity of grain

Grain dry weight/ main stem ear was calculated using the following formula-

Grain dry weight/ ear = Maximum ear dry weight – Ear dry weight at anthesis

Plant height

Plant height was measured from the base of the plant to the tip of the spike excluding the awn. Ten plants sample from each treatment were taken and means were calculated.

Spike character

Ten spikes from the main shoot were collected randomly per plot to collect spike characters data. Spike length (excluding awn) was measured manually using scale and number of kernels per spike was counted manually.

Number of spikes, grain and straw dry wt/m^2

The samples were collected from an area of 2m X 1m from the center of each plots by cutting the plant at ground level. Then ears were counted and collected in a cloth bag (2′ X 1.5′). The samples were dried in sun, threshed and cleaned manually and fresh weight of grain was taken. The husk, straw and representative samples of grain were dried in an oven at 70^0C for 72 hours to obtain grain and straw dry weight. Total dry weight/m^2 was calculated by adding grain, husk and straw dry weight.

Individual kernel size

From each plot thousand grains were taken randomly from dried sample and weighed. From this weight, average individual kernel size was calculated.

Biological yield, straw yield and grain yield

The biological yield, straw yield and grain yield were expressed in t/ha, grain yield also adjusted to 12% moisture.

Relative performance

The relative performance was calculated as Asana and Williams (1965) by the following formula-

$$\text{Relative performance} = \frac{\text{Variable measured under stress condition}}{\text{Variable measured under normal condition}}$$

Determination of flag leaf and grain N

Flag leaves from all experimental units at 80 days after sowing and harvested grain sample were collected for nitrogen determination and were kept in oven at 70^0C for 72 hours. The oven dried flag leaves and grain samples were ground and total nitrogen content was determined by Kjeldahl method as follows-

Digestion: 0.1g of plant sample was taken in a 100 ml Kjeldahl flask. 0.5g of catalyst (K_2SO_4 : $CuSO_45H_2O$: Selenium powder = 10:1:0.1) and 4 ml of 3% salicylicsulfuric acid were added in it and the flask was swirled until the acid and catalyst were mixed with the sample. Then the flask was heated continuously on a burner stand inside the fume hood. When the colour of the mixture had turned green, the mixture was boiled further for 30 minutes. The digest was cooled and 40 ml of distilled water was added in it. Then the solution was transferred in to 100 ml volumetric flask. After cooling the solution was diluted up to 100 ml with distilled water.

Distillation: 10 ml of plant digest from stock solution and 5 ml of 40% NaOH solution were poured in to the distillation chamber through funnel and the funnel was washed with small amount of distilled water. 5 ml of 4% boric acid indicator solution was taken in a 100 ml conical flask and was placed under the condenser of the distillation apparatus to collect the distillate. When the distillate reached to a volume of about 50 ml the distillation was stopped.

Titration: The distillate was titrated against 0.02N H_2SO_4 with the help of microburate. A blank titration was carried out following the same procedure of digestion and distillation.

%N was calculated by the following formula-

$$\%N = 14.007 \times N \text{ of } H_2SO_4 \times f \times (T - B) \times a/c \times p/w$$

Where,

14.007 for 1N H_2SO_4 (1ml) ≡ 14.007 mg of NH_4-N

N of H_2SO_4 used in titration = 0.02

f = correction factor = 1.02

T = Amount of 0.02N of H_2SO_4 (ml) required for sample titration

B = Amount of 0.02N of H_2SO_4 (ml) required for blank titration

a = Volume of the digest in volumetric flask (ml) = 100

c = Volume of the digest taken for distillation (ml) = 10

p = Conversion factor for percentage = 100

w = Dry weight of the plant sample taken for digestion in mg = 100

Statistical analysis

The findings were analyzed by partitioning the total variance with the help of computer by using MSTAT program. The treatment means were compared using Duncun's Multiple Range Test (DMRT) at 5% level of significance.

RESULTS AND DISCUSSION

A field experiment was conducted to evaluate the influence of application of extra nitrogen and/or splitting of nitrogen fertilizer after anthesis to sustain wheat yield due to late planting heat stress. In this experiment two sink sensitive heat tolerant (Kanchan and Fang 60), one sink insensitive heat tolerant (BAW 1059) and one heat sensitive (Sonora) wheat genotypes selected from experiment -3 were used as study material. To expose the wheat genotypes in to normal growing temperature and natural heat stress during reproductive development, all wheat genotypes were sown in the field on November 30 and December 30. In the present study, late sown (December 30) wheat genotypes exposed to heat stress (>26°C) during most of their reproductive growth phase and regarded as post anthesis heat stress condition but the wheat genotypes sown on November 30 experienced less than 26°C throughout their whole reproductive growth phase and considered as normal growing condition (Fig. 6.1). Because, the optimum temperature for reproductive stage of wheat lies a range of 22 to 26°C (Campbell and Read, 1968). Temperature above 26°C at reproductive growth phase causes harmful premature ripening of wheat (Aborl *et al.*, 1991).Results of the present study have been presented and discussed in this part.

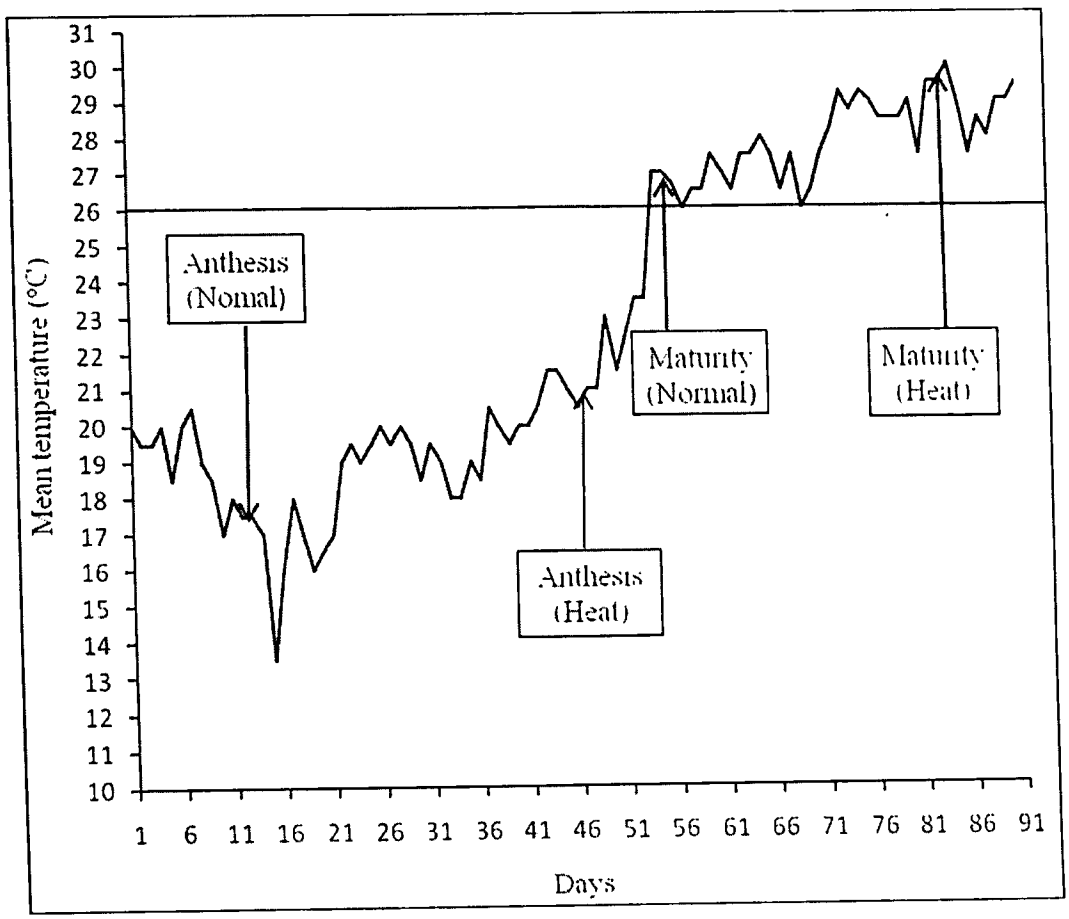


Fig. 6.1. Mean (mean of maximum and minimum) air temperature from 16 January to 15 April 2008 showing the period of anthesis and maturity of normal and late planting wheat.

Flag leaf nitrogen

Wheat genotypes and different nitrogen management under both growing conditions interacted significantly in flag leaf nitrogen content at 10 days after anthesis (Table 6.1). In Kanchan, flag leaf nitrogen concentration was significantly the highest (3.31%) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing condition (T_1) compared to all other nitrogen management under post anthesis heat stress condition. Application of RDNF with four splits under heat stress (T_3) showed the lowest uptake (2.80%) which was statistically identical with T_2 and T_5 in flag leaf N uptake. Addition of 50% extra N with three splits (T_4) significantly increased flag leaf N uptake (3.14%) compared to all other nitrogen management under stress condition i. e. T_2, T_3 and T_5.

In Fang 60, flag leaf nitrogen concentration was significantly the highest (3.66%) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing condition (T_1) compared to all other nitrogen management treatment under heat stress condition. Application of RDNF with four splits under heat stress (T_3) showed significantly the lowest uptake (2.94%) which was followed by the N uptake (3.23%) when RDF was applied with three splits under heat stress (T2). Addition of 50% extra N with three splits (T_4) or four splits (T5) increased flag leaf N uptake compared to T_2 but the increment was not significant.

Flag leaf nitrogen concentration in BAW 1059 was significantly the highest (3.43%) when RDNF was applied with three splits under normal growing condition (T_1) and it was (2.74%) significantly the lowest when RDNF was applied with three splits under heat stress growing condition (T_2) compared to all other nitrogen management treatment. Application of RDNF with four splits under heat stress (T_3) and addition of

50% extra N with three splits (T_4) or four splits (T5) significantly increased flag leaf N uptake compared to T_2.

In wheat genotypes Sonora, flag leaf nitrogen concentration was significantly the highest (3.40%) when RDF was applied with three splits under normal growing condition (T_1) and it was (2.63%) significantly the lowest when RDNF was applied with three splits under heat stress growing condition (T_2) compared to all other nitrogen management treatment. Application of RDNF with four splits under heat stress (T_3) and addition of 50% extra N with three splits (T_4) or four splits (T5) significantly increased flag leaf N uptake compared to T_2 but the increment was greater with T_4.

Figure 6.2 which shows the flag leaf nitrogen as % of normal indicated that the flag leaf nitrogen reduced in all wheat genotypes when RDNF was applied with three splits under heat stress growing condition (T_2) compared to that when RDNF was applied with three splits under normal growing condition (T_1). Application of RDNF as four splits under heat stress (T_3) could influence the flag leaf N only in BAW 1059 and Sonora. Application of additional 50% N either as three or four splits could increase the flag leaf N in all wheat genotypes but three splits (T_4) was more effective than four splits (T_5).

From the overall results it was clear that flag leaf nitrogen decreased significantly in all heat stressed wheat genotypes compared to those grown at normal growing temperature. Woolenweber *et al.* (2003) also reported that nitrogen contents in leaves reduces by 10% when wheat plants experience heat stress at the double ridge stage and by 25% at anthesis stage. Reduced flag leaf nitrogen under high temperature condition might be due to higher volatilization of N fertilizers such as NH_3

Table 6.1. Flag leaf nitrogen (%) at 10 days after anthesis of different wheat genotypes as influenced by different nitrogen managements at normal and heat stress conditions

Genotypes	Nitrogen managements	Flag leaf N (%)
Kanchan	T_1-RDNF with three splits (Normal)	3.31 cd
	T_2-RDNF with three splits (Heat)	2.83 i
	T_3-RDF with four splits (Heat)	2.80 i
	T_4-RDNF+ 50% with three splits (Heat)	3.14 ef
	T_5-RDNF+ 50% with four splits (Heat)	2.83 i
Fang 60	T_1-RDNF with three splits (Normal)	3.66 a
	T_2-RDNF with three splits (Heat)	3.23 de
	T_3-RDNF with four splits (Heat)	2.94 h
	T_4-RDNF+ 50% with three splits (Heat)	3.26 d
	T_5-RDNF+ 50% with four splits (Heat)	3.26 d
BAW 1059	T_1-RDNF with three splits (Normal)	3.43 b
	T_2-RDNF with three splits (Heat)	2.74 i
	T_3-RDNF with four splits (Heat)	3.03 gh
	T_4-RDNF+ 50% with three splits (Heat)	3.09 fg
	T_5-RDNF+ 50% with four splits (Heat)	2.97 h
Sonora	T_1-RDNF with three splits (Normal)	3.40 bc
	T_2-RDNF with three splits (Heat)	2.63 j
	T_3-RDNF with four splits (Heat)	2.77 i
	T_4-RDNF+ 50% with three splits (Heat)	2.97 h
	T_5-RDNF+ 50% with four splits (Heat)	2.8.0 i
CV(%)		1.77

In a column. values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

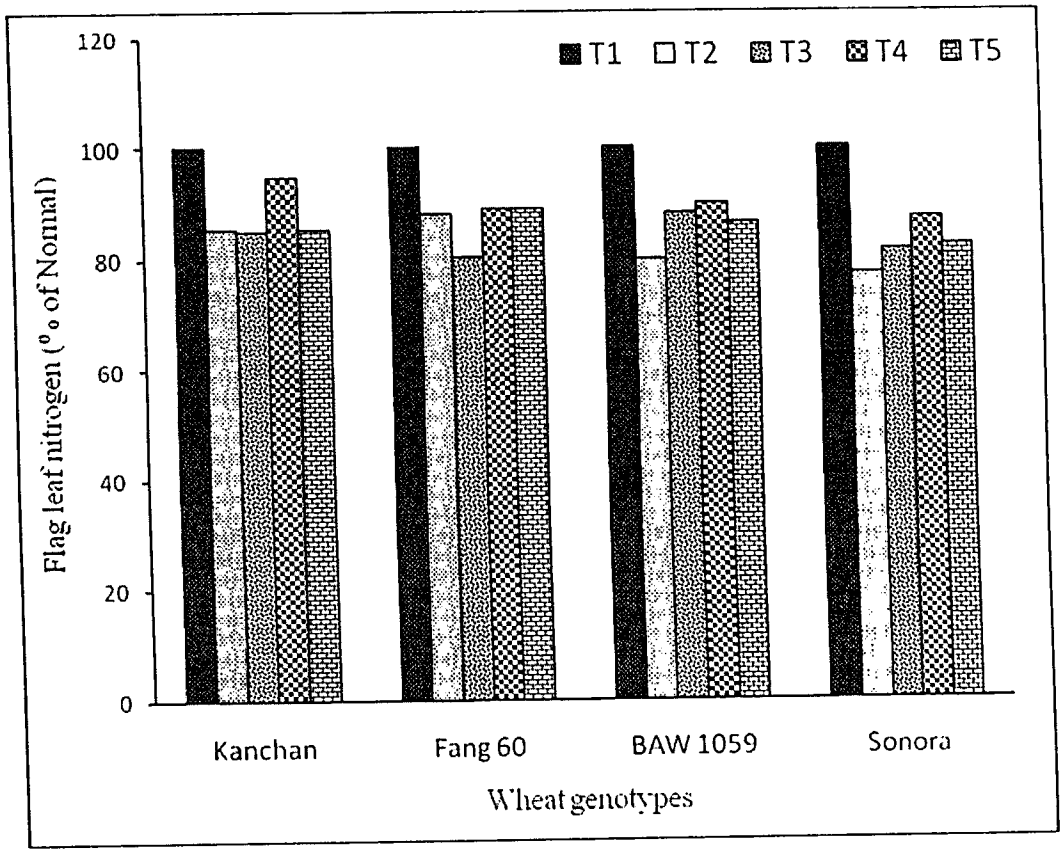


Fig. 6.2. Flag leaf nitrogen (% of Normal) at 10 days after anthesis of different wheat genotypes as influenced by different nitrogen managements at normal and heat stress conditions.

T_1-RDNF with three splits (Normal); T_2-RDNF with three splits (Heat)
T_3-RDNF with four splits (Heat); T_4-RDNF+ 50% with three splits (Heat)
T_5-RDNF+ 50% with four splits (Heat)

(Tran-Thuc-Son *et al.*, 1995). Application of additional 50% nitrogen fertilizer under heat stress condition increased the N uptake more or less in all wheat genotypes. Malhi and Kutcher (2004) also found increased N uptake with increasing N application. The difference in flag leaf nitrogen conten in different nitrogen management indicated the effectiveness of different treatments applied in the present study.

Flag leaf senescence

The sharp decrease in SPAD value occurred at a SPAD reading between 25 and 30 indicated the onset of leaf senescence. The SPAD value taken from the middle portion of flag leaf was the maximum at or near anthesis in all wheat genotypes with different nitrogen management at normal and heat stress condition. The SPAD value was found to be decreased slowly during early post anthesis stage and later a rapid decrease in this value occurred at a SPAD reading in between 25 and 30 (Fig. 6.3).

Under normal growing condition when recommended dose of nitrogen fertilizer was applied in three splits, the rapid decrease in SPAD value that in other words onset of flag leaf senescence occurred at 30 DAA in heat tolerant genotypes (Kanchan, Fang 60, and BAW 1059) and 28 DAA in heat sensitive Sonora. Due to different nitrogen management treatments at post anthesis heat stress condition, onset of flag leaf senescence occurred 8 day earlier in HT Kanchan & Fang 60, 10 days in another HT genotype (BAW 1059) and 10-12 days in heat sensitive Sonora. Application of RDNF + extra 50% N fertilizer under heat stress condition as three splits increased the flag leaf longevity for 2 days in Kanchan and 4 days in BAW 1059.

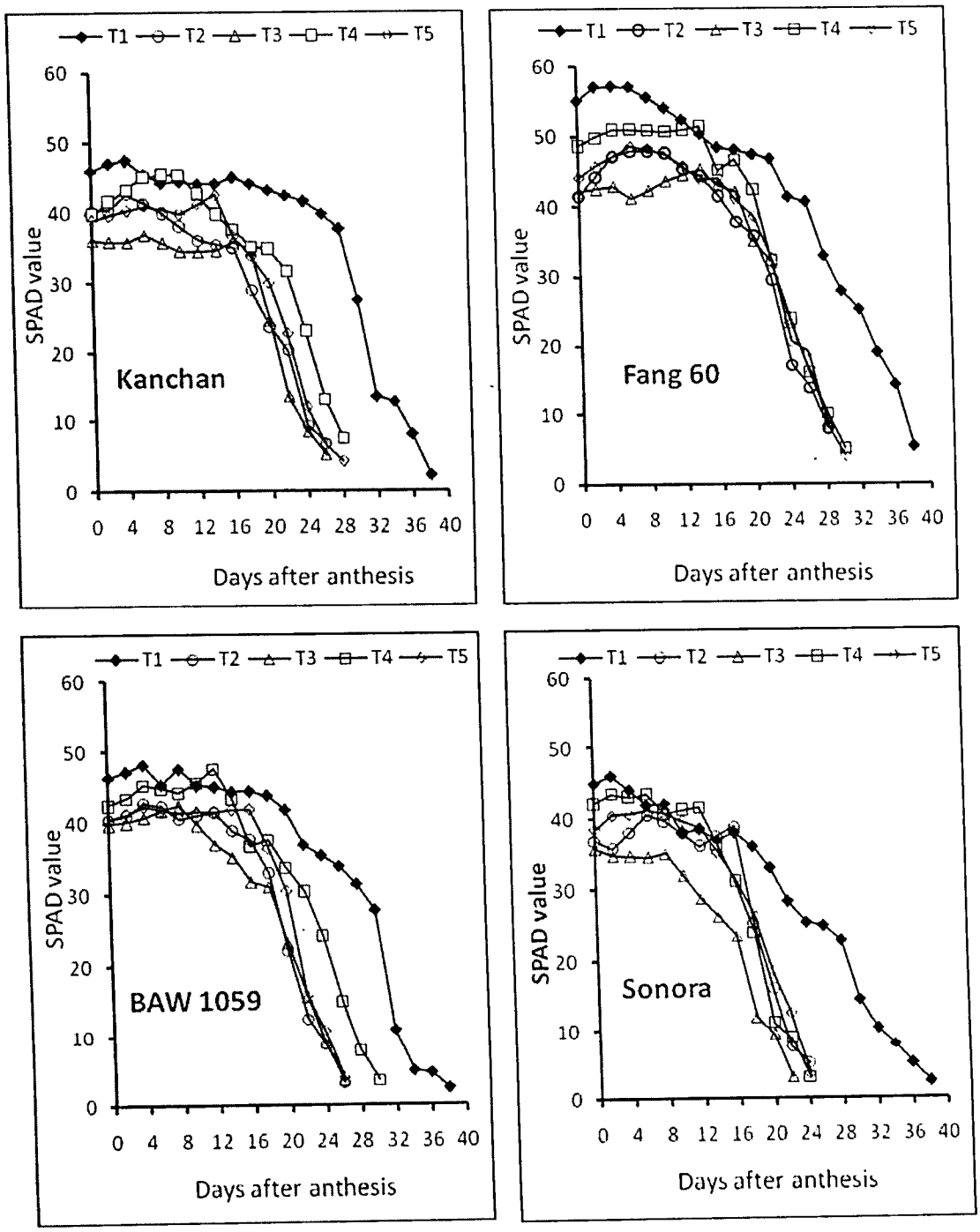


Fig. 6.3. SPAD value of different wheat genotypes as influenced by different nitrogen managements at normal and heat stress conditions (Each point is the mean of three replicates).

T_1-RDNF with three splits (Normal); T_2-RDNF with three splits (Heat)
T_3-RDNF with four splits (Heat); T_4-RDNF+ 50% with three splits (Heat)
T_5-RDNF+ 50% with four splits (Heat)

Possible reasons of early onset of flag leaf senescence due to terminal heat stress may be increased activity of peroxidase (Jiang *et al.*, 2007), increase in cholorophyll breakdown (Thomas *et al.*, 1980), Inactivation of oxygen evolving complex of PSII and chlophyll protein complex (Ferguson *et al*, 1993), inactivation of Rubisco (Grover *et al.*, 1986), reduction in chlorophyll biosynthesis, decreased level of cytokinin as a result of heat shock, loss of membrane integrity due to excessive fluidity of membrane lipid and, denaturation of membrane bound H^+ pumping ATPase (Ranjan *et al.*, 2001). The heat tolerant wheat genotypes showed somewhat tolerance against heat stress induced flag leaf senescence. This may be due to the amelioration of high temperature induced oxidative stress by antioxidant enzymes like superoxide dismutase and catalase (Almeselmani *et al.*, 2006).

Stem reserve utilization

The combine effect of wheat genotypes and different nitrogen management at different growing temperature on main stem reserve utilization was significant (Table 6.2). In Kanchan, stem reserve utilization was the lowest (0.475 g/mainstem) when recommended dose of nitrogen fertilizer was applied with three splits under heat stress (T_2) compared to all other nitrogen management treatment which was statistically similar to those obtained from T_3, T_4 and T_1. Application of RDNF + extra 50% N fertilizer with four splits at heat stress showed the highest mainstem reserve utilization (0.577 g/mainstem) which was statistically similar to that obtained from RDF with three splits at normal condition (T_1) but dissimilar with all other nitrogen management treatments. The relative value showed that the treatment T_3 (0.91) and T_5 (1.05) positively influenced the mainstem reserve utilization over the treatment T_2. (0.87).

In Fang 60, stem reserve utilization was the lowest (0.419 g/mainstem) when recommended dose of nitrogen fertilizer was applied with three splits under heat stress (T_2) compared to all other nitrogen management treatment which was statistically similar to those obtained from T_4 and T_5. Application of RDNF fertilizer with four splits at heat stress (T_3) showed the highest mainstem reserve utilization (0.542 g/mainstem) which was statistically similar to that (0.519 g/mainstem) obtained from RDF with three splits at normal condition (T_1). The relative value showed that the treatment T_3 (1.04), T_4 (0.90) and T_5 (0.88) positively influenced the mainstem reserve utilization over the treatment T_2 (0.81).

In BAW 1059, mainstem reserve utilization was the lowest (0.432 g/mainstem) when recommended dose of nitrogen fertilizer was applied with four splits under heat stress (T_3) compared to all other nitrogen management treatment. Application of RDNF +50% extra N fertilizer with three splits at heat stress (T_4) showed the highest mainstem reserve utilization (0.634 g/mainstem) which was statistically similar to those obtained from T_1 (0.595 g/mainstem), T_2 (0.592 g/mainstem) and T_5 (0.597 g/mainstem). The relative value showed that the treatment T_4 (1.07) and T_5 (1.00) positively influenced the mainstem reserve utilization over the treatment T_2 (0.99).

In Sonora, mainstem reserve utilization was significantly the highest (0.482 g/mainstem) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) compared to all other nitrogen management treatment. Application of RDNF +50% extra N fertilizer with four splits at heat stress (T_5) showed the lowest mainstem reserve utilization (0.310 g/mainstem) which was statistically similar to those obtained from T_2 (0.311 g/mainstem), T_3 (0.312 g/mainstem) and T_4 (0.390 g/mainstem). The relative value showed that the

Table 6.2. Stem reserve utilization of different wheat genotypes as influenced by different nitrogen managements at normal and heat stress condition

Genotypes	Nitrogen managements	Stem reserve utilization	
		Actual (g/main stem)	Relative to T_1
Kanchan	T_1-RDNF with three splits (Normal)	0.547 bc	-
	T_2-RDNF with three splits (Heat)	0.475 ce	0.87
	T_3-RDNF with four splits (Heat)	0.498 ce	0.91
	T_4-RDNF+ 50% with three splits (Heat)	0.485 ce	0.87
	T_5-RDNF+ 50% with four splits (Heat)	0.577 bd	1.05
Fang 60	T_1-RDNF with three splits (Normal)	0.519 bd	-
	T_2-RDNF with three splits (Heat)	0.419 ef	0.81
	T_3-RDNF with four splits (Heat)	0.542 bd	1.04
	T_4-RDNF+ 50% with three splits (Heat)	0.467 cf	0.90
	T_5-RDNF+ 50% with four splits (Heat)	0.457 df	0.88
BAW 1059	T_1-RDNF with three splits (Normal)	0.595 ab	-
	T_2-RDNF with three splits (Heat)	0.592 ab	0.99
	T_3-RDNF with four splits (Heat)	0.432 ef	0.72
	T_4-RDNF+ 50% with three splits (Heat)	0.634 a	1.07
	T_5-RDNF+ 50% with four splits (Heat)	0.597 ab	1.00
Sonora	T_1-RDNF with three splits (Normal)	0.482 ce	-
	T_2-RDNF with three splits (Heat)	0.311 g	0.65
	T_3-RDNF with four splits (Heat)	0.312 g	0.65
	T_4-RDNF+ 50% with three splits (Heat)	0.390 fg	0.81
	T_5-RDNF+ 50% with four splits (Heat)	0.310 g	0.64
CV(%)		9.64	-

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

treatment T_4 (0.81) positively influenced the mainstem reserve utilization over the treatment T_2 (0.99).

The overall results in mainstem reserve utilization indicated that BAW 1059 showed the highest reserve utilization compared to other wheat genotypes. It also maintained higher mainstem reserve utilization under stress condition. Addition of RDNF + extra 50% N with three splits influenced the reserve utilization further in BAW 1059. Increased stem reserve utilization under heat stress with additional 50% N fertilizer might be due better early growth of wheat plant (Badaruddin *et al.* 1999). Greater ability of the grain of BAW 1059 to utilize current or reserve photosynthate under heat stress also contributed to increase in main stem reserve utilization under post anthesis heat stress condition.

Grain nitrogen

Wheat genotypes and different nitrogen management under both growing conditions interacted significantly in grain nitrogen content at maturity (Table 6.3). In Kanchan, grain nitrogen concentration was the lowest (2.86%) when recommended dose of nitrogen fertilizer was applied with four splits under heat stress (T_3) compared to all other nitrogen management which was statistically similar to that (2.89%) obtained from RDNF with three splits under post anthesis heat stress condition (T_2). The grain nitrogen content obtained from RDNF with three splits under normal condition followed the grain N uptake by the treatment T_3. Addition of 50% extra N with three splits (T_4) and four splits (T_5) at stress condition significantly increased grain N uptake (3.17%) compared to all other nitrogen management.

In Fang 60, grain nitrogen concentration was the lowest (2.54%) when recommended dose of nitrogen fertilizer was applied with four splits under heat stress (T_3) compared

to all other nitrogen management which was followed by those obtained from RDNF with three splits at normal (2.71%) and post anthesis heat stress condition (2.77%). Addition of 50% extra N with three splits (T_4) and four splits (T_5) at stress condition significantly increased grain N uptake (2.97%) compared to all other nitrogen management.

Grain nitrogen concentration in wheat genotype BAW 1059, was the lowest (3.11%) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing condition (T_1) compared to all other nitrogen management which was statistically similar to that (3.17%) obtained from RDNF with four splits at heat stress condition (T_3). The grain nitrogen content (3.37%) obtained from RDF with three splits under heat stress condition (T_2) followed the grain N uptake by the treatment T_3 and there was significant difference between them in grain N uptake. Addition of 50% extra N with three splits (T_4) and four splits (T_5) at heat stress condition significantly increased grain N uptake compared to all other nitrogen management but the increment was greater in T_5 (4.03%) than T_4 (3.71%).

In wheat genotype Sonora, grain nitrogen concentration was the lowest (3.80%) when recommended dose of nitrogen fertilizer was applied with three splits under heat stress (T_2) compared to all other nitrogen management which was statistically similar to that (3.86%%) obtained from RDNF with three splits under normal growing condition (T_1). The grain nitrogen content (4.09%) obtained from RDF with four splits under post anthesis heat stress condition followed the grain N uptake by the treatment T_3. Addition of 50% extra N with three splits (T_4) and four splits (T_5) at stress condition further increased grain N uptake (4.26%) significantly compared to all other nitrogen management.

Table 6.3. Grain nitrogen (%) at maturity of different wheat genotypes as influenced by different nitrogen managements at normal and heat stress conditions

Genotypes	Nitrogen managements	Grain N (%)
Kanchan	T_1-RDNF with three splits (Normal)	3.09 fg
	T_2-RDNF with three splits (Heat)	2.89 hi
	T_3-RDF with four splits (Heat)	2.86 hi
	T_4-RDNF+ 50% with three splits (Heat)	3.17 f
	T_5-RDNF+ 50% with four splits (Heat)	3.17 f
Fang 60	T_1-RDNF with three splits (Normal)	2.71 j
	T_2-RDNF with three splits (Heat)	2.77 ij
	T_3-RDNF with four splits (Heat)	2.54 k
	T_4-RDNF+ 50% with three splits (Heat)	2.97 h
	T_5-RDNF+ 50% with four splits (Heat)	2.97 h
BAW 1059	T_1-RDNF with three splits (Normal)	3.11 f
	T_2-RDNF with three splits (Heat)	3.37 e
	T_3-RDNF with four splits (Heat)	3.17 f
	T_4-RDNF+ 50% with three splits (Heat)	3.71 d
	T_5-RDNF+ 50% with four splits (Heat)	4.03 b
Sonora	T_1-RDNF with three splits (Normal)	3.86 c
	T_2-RDNF with three splits (Heat)	3.80 cd
	T_3-RDNF with four splits (Heat)	4.09 b
	T_4-RDNF+ 50% with three splits (Heat)	4.26 a
	T_5-RDNF+ 50% with four splits (Heat)	4.26 a
CV(%)		2.01

In a column. values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

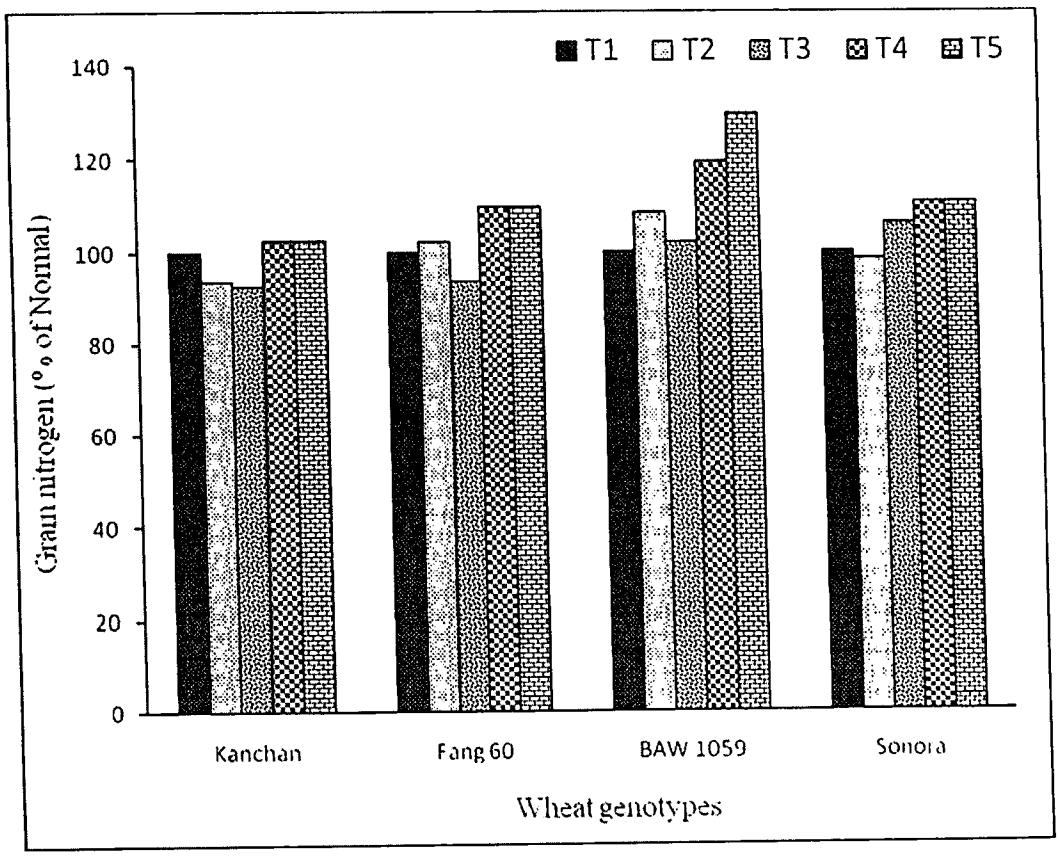


Fig. 6.4. Grain nitrogen (% of Normal) at maturity of different wheat genotypes as influenced by different nitrogen managements at normal and heat stress conditions.

T_1-RDNF with three splits (Normal); T_2-RDNF with three splits (Heat)
T_3-RDNF with four splits (Heat); T_4-RDNF+ 50% with three splits (Heat)
T_5-RDNF+ 50% with four splits (Heat)

Araus *et al.* (1986) and Chowdhury & Wardlaw (1978) reported that percentage nitrogen in kernel increased with increasing temperature. This result partially supports the result of the present study. Application of additional 50% nitrogen fertilizer under heat stress condition increased the N uptake by grain in all wheat genotypes. Malhi and Kutcher (2004) also found increased N uptake with increasing N application.

Figure 6.4 which shows the grain nitrogen as % of normal indicated that the grain nitrogen reduced in Kanchan and Sonora when RDNF was applied with three splits under heat stress growing condition (T_2) compared to that when RDNF was applied with three splits under normal growing condition (T_1) but in Fang 60 and Baw 1059 it was even increased. Application of RDNF as four splits under heat stress (T_3) influenced the grain N only in BAW 1059 and Sonora. Application of additional 50% N either as three or four splits increased the grain N in all wheat genotypes. The overall results in grain N (% of Normal) showed that BAW 1059 maintained comparatively higher grain N with additional 50% nitrogen under heat stress condition than other wheat genotypes which indicated greater sink activity of BAW 1059 at later reproductive development under heat stressed condition.

Ear dry matter accumulation

Different nitrogen management treatments at normal and heat stress condition showed a typical sigmoid pattern of dry matter accumulation in ear in all wheat genotypes (Fig. 6.5 and 6.6). In Kanchan, the ear dry weight was observed to be increased up to 2.455 g at 40 days after anthesis (DAA) when RDNF was applied with three splits under normal growing temperature (T_1). Due to different nitrogen management treatments under post anthesis heat stress condition, maximum dry matter accumulation in ear and days required to attain maximum dry weight were reduced.

The ear dry weight increased up to 2.115 g at 32 DAA with treatment T_2, 1.943 g at 32 DAA with T_3, 2.315g at 36 DAA with T_4 and 2.199g at 36 DAA with T_5.

In Fang 60, maximum dry matter accumulation in ear (2.575g) was observed at 40 DAA when RDNF was applied with three splits under normal growing temperature (T_1). Different nitrogen management treatments at post anthesis heat stress condition reduced the maximum dry matter accumulation in ear and the days required to attain maximum dry weight. The maximum dry weight accumulation was 2.457g at 32 DAA with treatment T_2, 2.243g at 24 DAA with T_3, 2.463g at 32 DAA with T_4 and 2.33g at 28 DAA with T_5.

In BAW 1059, the ear dry weight was observed to be increased up to 2.83g at 36 DAA when RDNF was applied with three splits under normal growing temperature (T_1). Due to different nitrogen management treatments under post anthesis heat stress condition, maximum dry matter accumulation in ear and days required to attain maximum dry weight were reduced. The ear dry weight increased up to 2.733 g at 28 DAA with treatment T_2, 2.817g at 28 DAA with T_3, 2.82g at 32 DAA with T_4 and 2.553g at 24 DAA with T_5.

In Sonora, maximum dry matter accumulation in ear (2.196g) was observed at 40 DAA when RDNF was applied with three splits under normal growing temperature (T_1). Different nitrogen management treatments at post anthesis heat stress condition reduced the maximum dry matter accumulation in ear and the days required to attain maximum dry weight. Only 24 days was required to attain maximum dry weight due to different nitrogen management treatments under post anthesis heat stress condition. The maximum dry weight accumulation was 1.926g with treatment T_2, 1.907g with T_3, 2.013g with T_4 and 1.917g with T_5.

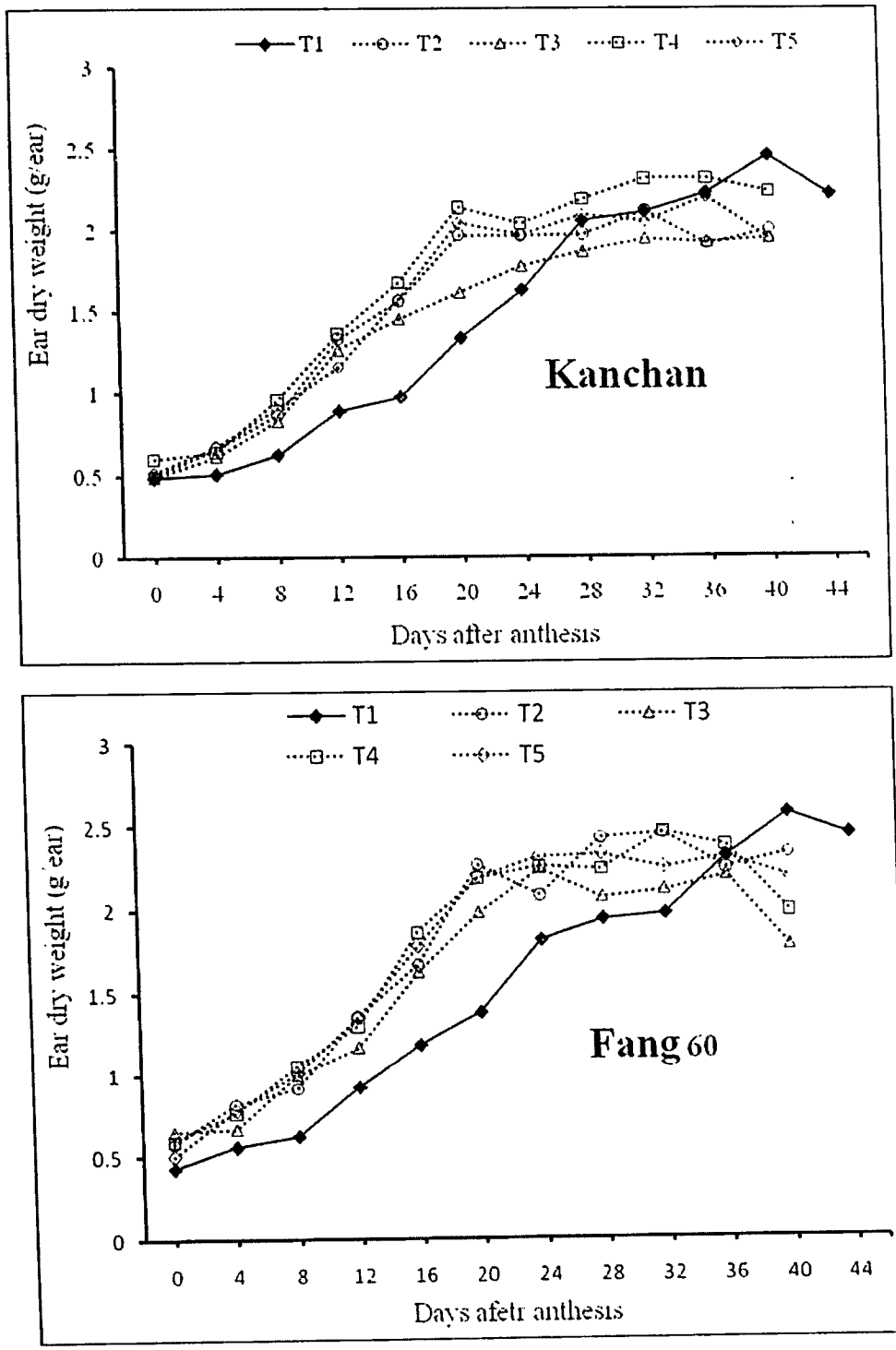


Fig. 6.5. Ear dry weight of different wheat genotypes (Kanchan and Fang 60) at different days after anthesis as influenced by different nitrogen managements at normal and heat stress condition (Each point is the mean of three replicates).

T_1-RDNF with three splits (Normal); T_2-RDNF with three splits (Heat)
T_3-RDNF with four splits (Heat); T_4-RDNF+ 50% with three splits (Heat)
T_5-RDNF+ 50% with four splits (Heat)

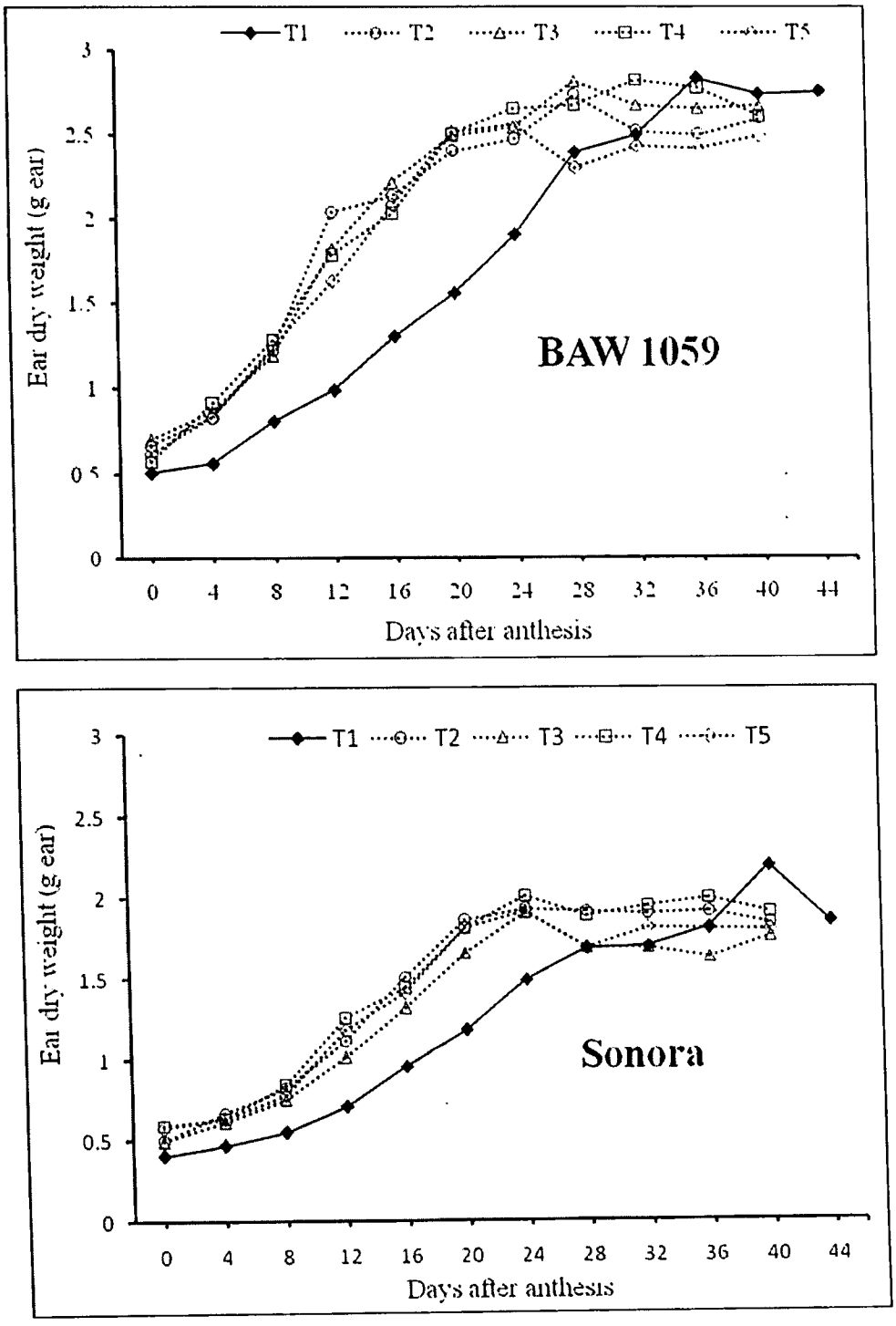


Fig. 6.6. Ear dry weight of different wheat genotypes (BAW 1059 and Sonora) at different days after anthesis as influenced by different nitrogen managements at normal and heat stress condition (Each point is the mean of three replicates).

T_1-RDNF with three splits (Normal); T_2-RDNF with three splits (Heat)
T_3-RDNF with four splits (Heat); T_4-RDNF+ 50% with three splits (Heat)
T_5-RDNF+ 50% with four splits (Heat))

The overall result indicated that application of RDNF + extra 50% N fertilizer with three splits (T_4) increased ear dry matter accumulation in in all wheat genotypes compared to that obtained from application of RDNF with three splits at heat stress condition (T_2) but the increment was greater in HT genotypes (Kanchan, Fang 60 and BAW 1059) than HS genotype (Sonora). Application of RDNF + extra 50% N fertilizer with three splits (T_4) increased the ear growth duration also in Kanchan and BAW 1059 compared to that obtained from T_2.

Grain dry weight per main stem ear

The combine effect of wheat genotypes and different nitrogen management at different growing temperature on main stem grain dry weight was significant (Fig. 6.7). In Kanchan, grain dry weight per main stem ear was significantly the highest (1.965g) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) compared to all nitrogen management treatments under heat stress condition. Grain dry weight per main stem ear was significantly the lowest (1.451g) when RDNF was applied with four splits under heat stress condition (T_3) which was followed by those obtained from treatments T_4 (1.715g), T_5 (1.679g) and T_2 (1.662g). Application of RDNF + extra 50% N fertilizer with three splits at heat stress (T_4) showed increased grain dry weight per main stem ear to some extend over RDNF with three splits under heat stress (T_2).

In Fang 60, grain dry weight per main stem ear was significantly the lowest (1.597g) when recommended dose of nitrogen fertilizer was applied with four splits under heat stress (T_3) compared to all other nitrogen management treatment which was followed by those obtained from T_5 (1.829g), T_4 (1.871g) and T_2 (1.885g). Application of

RDNF fertilizer with three splits at normal growing condition (T_1) showed significantly the highest grain dry weight per main stem ear (2.145g).

In BAW 1059, grain dry weight per main stem ear was the lowest (2.001g) when RDNF + extra 50% N fertilizer was applied with four splits under heat stress (T_5) which was statistically similar to those obtained from T_2 (2.069g) and T_3 (2.117g). Application of RDNF fertilizer with three splits at normal growing condition (T_1) showed significantly the highest grain dry weight per main stem ear (2.326g) which was statistically at par with that (2.252g) obtained from RDNF + extra 50% N fertilizer with three splits under heat stress (T_4). Here Application of RDNF + extra 50% N fertilizer with three splits at heat stress (T_4) showed significant increase in grain dry weight per main stem ear over RDNF with three splits under heat stress (T_2).

In Sonora, grain dry weight per main stem ear was significantly the highest (1.788g) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) compared to all other nitrogen management treatment. Application of RDNF +50% extra N fertilizer with four splits at heat stress (T_5) showed the lowest grain dry weight per main stem ear (1.339g) which was statistically similar to those obtained from other nitrogen management treatment under heat stress condition.

The overall results in grain dry weight per main stem ear indicated that application of RDNF +50% extra N fertilizer with three splits at heat stress (T_4) showed increased grain dry weight per main stem ear compared to that obtained from RDNF with three splits under heat stress (T_2) in Kanchan and BAW 1059 but the increment was significant only in BAW 1059. This might be due to its greater ability to utilize current or reserve photosynthate and greater main stem reserve utilization under post anthesis heat stress condition.

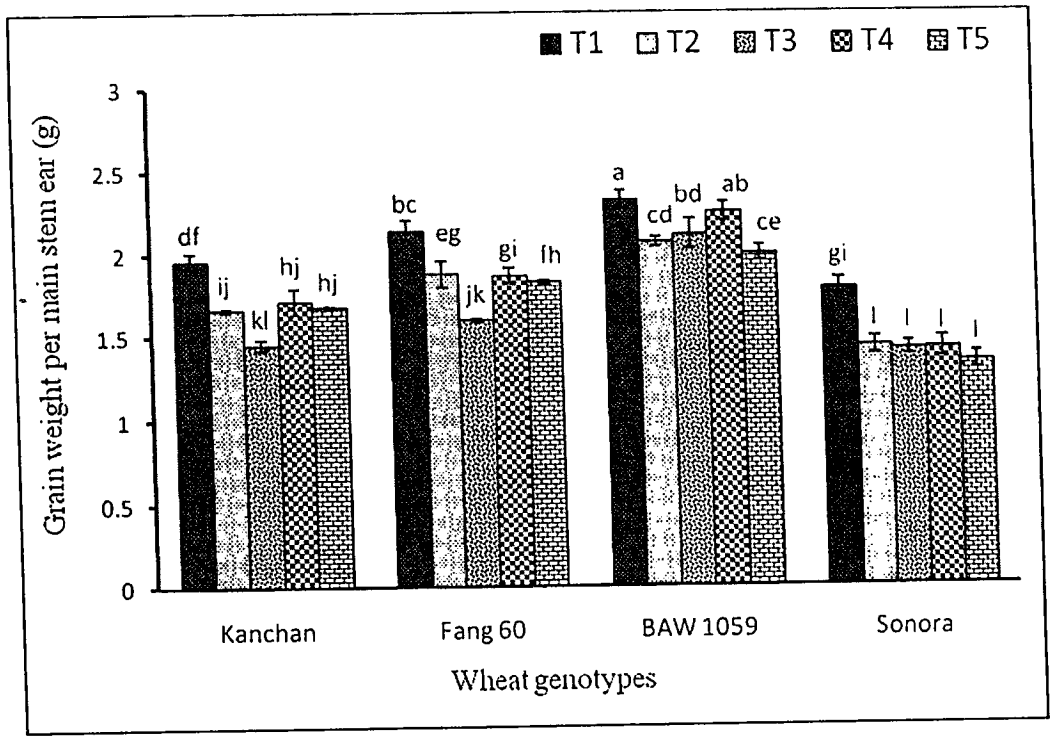


Fig. 6.7. Grain weight per main stem ear (mean ±SE) of different wheat genotypes as influenced by different nitrogen managements at normal and heat stress condition. Values followed by the different letters are significantly different from each other by DMRT at 5% level.

T_1-RDNF with three splits (Normal); T_2-RDNF with three splits (Heat)
T_3-RDNF with four splits (Heat); T_4-RDNF+ 50% with three splits (Heat)
T_5-RDNF+ 50% with four splits (Heat)

Plant height

Plant height was not influenced significantly by the interaction effect of wheat genotypes and different nitrogen management at normal and heat stress condition (fig. 6.8) though it was influenced significantly by wheat genotypes. Among the wheat genotypes tested, Sonora was the shortest (76.37cm) and other three wheat genotypes did not differ significantly in plant height. Badaruddin *et al.* (1999) also found that extra nitrogen did not influence the plant height under heat stress condition.

Ear length

The interaction effect of wheat genotypes and different nitrogen management at normal and heat stress condition did not influence spike length (fig. 6.9) though it was influenced significantly by wheat genotypes. Among the wheat genotypes tested, Sonora produced the shortest spike (9.57cm) and other three wheat genotypes did not differ significantly in spike length.

Number ears per m^2

The combine effect of wheat genotypes and different nitrogen management at different growing temperature influenced the number of ears per m^2 significant (Table 6.4). In Kanchan, ear number per m^2 was significantly the highest (408) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) compared to all nitrogen management treatments under heat stress condition. Ear number per m^2 was the lowest (280) when RDNF was applied with four splits under heat stress condition (T_3) which was statistically similar to those obtained from treatments T_2 (318), T_4 (348) and T_5 (334). Application of RDNF + extra 50% N fertilizer with three splits (T_4) and four splits (T_5) at heat stress showed

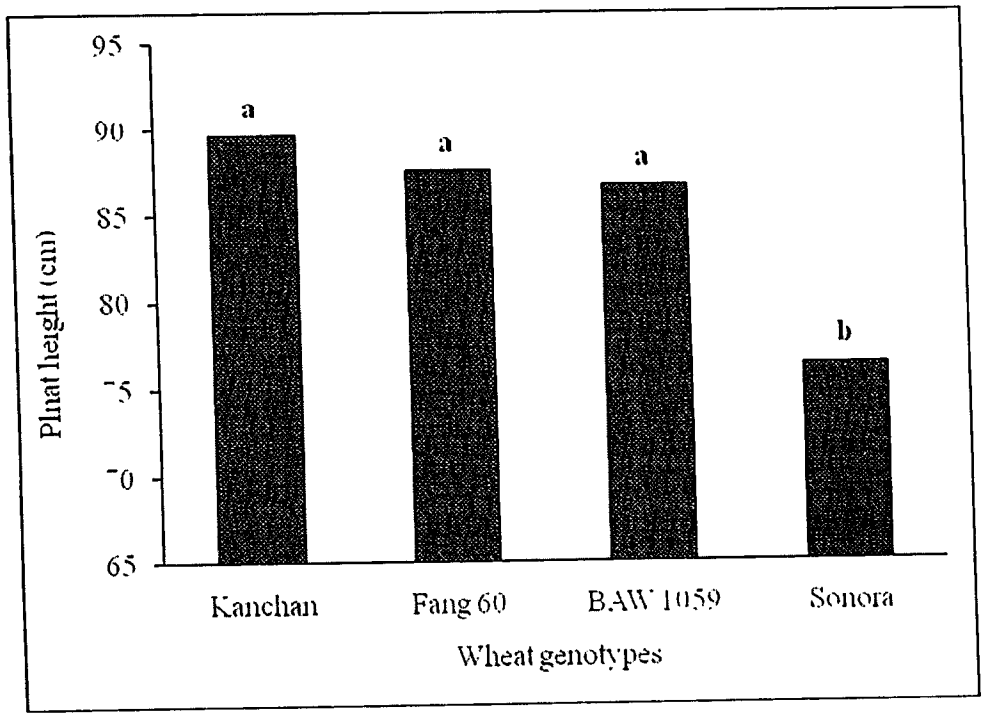


Fig. 6.8. Plant height of different wheat genotypes. Values followed by the different letters are significantly different from each other by DMRT at 5% level.

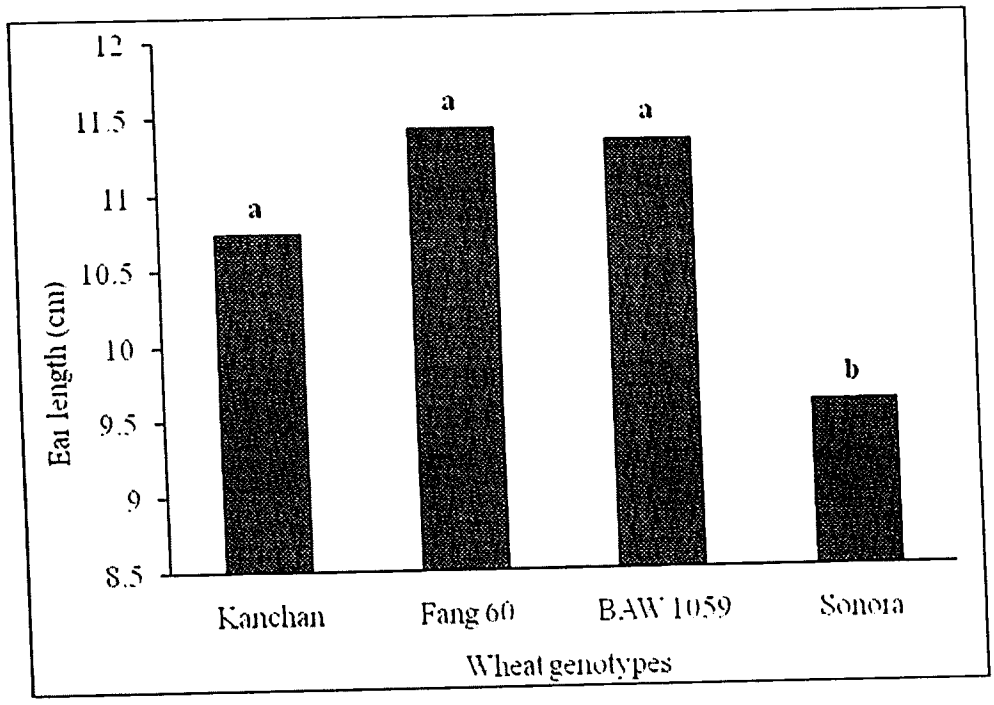


Fig. 6.9. Ear length of different wheat genotypes. Values followed by the different letters are significantly different from each other by DMRT at 5% level.

increased ear number per m^2 over RDNF with three splits under heat stress (T_2) though the increment was not significant.

In Fang 60, ear number per m^2 was significantly the highest (496) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) compared to all nitrogen management treatments under heat stress condition. Ear number per m^2 was the lowest (287) when RDNF was applied with four splits under heat stress condition (T_3) which was statistically similar to those obtained from treatments T_2 (307), T_4 (331) and T_5 (291). Application of RDNF + extra 50% N fertilizer with three splits (T_4) at heat stress showed increased ear number per m^2 over RDNF with three splits under heat stress (T_2) though the increment was not significant..

In BAW 1059, ear number per m^2 was significantly the highest (362) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) compared to all nitrogen management treatments under heat stress condition which was followed by that (267) obtained from RDNF + extra 50% N fertilizer with three splits (T_4). Ear number per m^2 was the lowest (213) when RDNF was applied with four splits under heat stress condition (T_3) which was statistically similar to those obtained from treatments T_2 (238 and T_5 (243). Application of RDNF + extra 50% N fertilizer with three splits (T_4) and four splits (T_5) at heat stress showed increased ear number per m^2 over RDNF with three splits under heat stress (T_2) though the increment was not significant..

In heat sensitive genotype Sonora, the highest ear number per m^2 (486) was obtained when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) compared to all other nitrogen management

treatment. Application of RDNF with four splits at heat stress (T_3) and application of RDNF + extra 50% with three splits at heat stress (T_4) showed the lowest ear number per m^2 (282) which was statistically similar to those obtained from T_2 (284) and T_3 (283).

The overall results in ear number per m^2 showed that application of RDNF +50% extra N fertilizer with three splits (T_4) and four splits (T_5) at heat stress showed increased ear number per m^2 compared to that obtained from RDNF with three splits under heat stress (T_2) in Kanchan and BAW 1059 but in Fang 60, ear number per m^2 increased only when RDNF +50% extra N fertilizer was applied with three splits (T_4). Badaruddin *et al.* (1999) found 6% increase in ear number/m^2 due to application of 50% extra N under heat stress condition. In sensitive genotype (Sonora), ear number per m^2 was not influenced by extra nitrogen treatments.

Number grains per ear

Wheat genotypes and different nitrogen management at normal and high temperature growing temperature interacted significantly to influence grain number per ear (Table 6.4). In Kanchan, grain number per ear was the lowest (34.7) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) compared to all nitrogen management treatments under heat stress condition but it was statistically similar to those obtained from the treatments T_3 (36.0) and T_5 (36.4) . Grain number per ear was the highest (44.5) when RDNF was applied with three splits under heat stress condition (T_2) which was statistically similar to that (42.7) obtained from application of RDNF + extra 50% N fertilizer with three splits (T_4).

In Fang 60, grain number per ear was the lowest (48.1) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1). Due to different nitrogen management at heat stress condition grain number per ear was increased. Application of RDNF + extra 50% N fertilizer with three splits (T_4) showed the highest grain number per ear (54.9) which was statistically similar to those obtained from T_2. Application of RDNF and RDNF +extra 50%N with four splits produced the moderate number of grain per ear (50.3).

In BAW 1059, grain number per ear was the lowest (35.7) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1). Due to different nitrogen management at heat stress condition grain number per ear was increased but the increment was significant only for T_5 (40.3).

In heat sensitive Sonora, grain number per ear was the highest (51.0) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1). Due to different nitrogen management at heat stress condition grain number per ear was decreased but the reduction was significant for T_3 and T_4.

The overall results in grain number per ear showed that application of RDNF +50% extra N fertilizer with three splits (T_4) and four splits (T_5) at heat stress showed increased grain number per ear compared to that obtained from RDNF with three splits under heat stress (T_2) in BAW 1059 but in Fang 60, grain number per ear increased only when RDNF +50% extra N fertilizer was applied with three splits (T_4). In sensitive genotype (Sonora), grain number per ear was influenced by extra nitrogen treatments when applied with four splits (T_5).

Grain size

The combine effect of wheat genotypes and different nitrogen management at different growing temperature influenced the grain size significant (Table 6.4). Wheat genotype Kanchan produced the significantly largest grain (38.87mg) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) and it produced significantly the smallest grain (28.92mg) when RDNF + extra 50% N fertilizer was applied with three splits at heat stress condition (T_4) compared to all other nitrogen management treatments. Application of RDNF with three splits at heat stress condition (T_2) provided the second largest grain (32.38mg) which was statistically similar to that (31.57mg) obtained from T_3 and dissimilar to that (30.36mg) obtained from T_5.

In Fang 60, significantly the largest grain (29.86mg) was produced when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) and significantly the smallest grain (22.82mg) was produced when RDNF + extra 50% N fertilizer was applied with three splits at heat stress condition (T_4) compared to all other nitrogen management treatments. The other nitrogen management treatments under heat stress produced moderate grain size and the grain produced by them was not differed significantly in size.

Wheat genotype BAW 1059 produced the significantly largest grain (50.22mg) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) which was followed by those produced from T_3 (46.46mg), T_2 (45.17mg) and T_4 (44.59mg). BAW 1059 produced significantly the smallest grain (43.60mg) when RDNF + extra 50% N fertilizer was applied with four splits at heat stress condition (T_5).

Table 6.4. Yield contributing characters of different wheat genotypes as influenced by different nitrogen managements at normal and heat stress condition

Genotypes	Nitrogen managements	Ear/m^2	No. of grain/ ear	Grain size (mg)
Kanchan	T_1	408 b	34.7 i	38.87 e
	T_2	318 ce	44.5 de	32.38 f
	T_3	280 df	36.0 gi	31.57 fg
	T_4	348 c	42.7 ef	28.92 i
	T_5	334 cd	36.4 gi	30.36 gh
Fang 60	T_1	496 a	48.1 bd	29.86 hi
	T_2	307 ce	51.2 ab	25.69 j
	T_3	287 df	50.3 bc	25.68 j
	T_4	331 cd	54.9 a	22.82 k
	T_5	291 df	50.3 bc	26.09 j
BAW 1059	T_1	362 bc	35.7 hi	50.22 a
	T_2	238 fg	37.3 gi	45.17 c
	T_3	213 g	39.6 fh	46.46 b
	T_4	267 ef	39.5 fh	44.59 cd
	T_5	243 fg	40.3 fg	43.60 d
Sonora	T_1	486 a	51.0 ab	30.48 gh
	T_2	284 df	48.8 bd	25.17 j
	T_3	282 df	46.0 ce	25.39 j
	T_4	282 df	46.4 ce	23.47 k
	T_5	283 df	50.0 bc	23.24 k
CV(%)		9.04	6.52	2.21

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

T_1-RDNF with three splits (Normal); T_2-RDNF with three splits (Heat)
T_3-RDNF with four splits (Heat); T_4-RDNF+ 50% with three splits (Heat)
T_5-RDNF+ 50% with four splits (Heat)

In heat sensitive Sonora, grain size was the largest (30.48mg) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1). Due to different nitrogen management at heat stress condition grain size was decreased. The smallest grain (23.24 mg) was produced when RDNF + extra 50% N fertilizer was applied with four splits at heat stress condition (T_5) which was statistically identical with that (23.47mg) obtained from T_4. Treatment T_2 and T_3 produced the moderate grain size.

Grain yield

The combine effect of wheat genotypes and different nitrogen management at normal and high temperature growing condition on grain yield was significant (Table 6.5). In Kanchan, grain yield was significantly the highest (451g/m^2) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) compared to all nitrogen management treatments under heat stress condition. Grain yield was significantly the lowest (224g/m^2) when RDNF was applied with four splits under heat stress condition (T_3) which was followed by those obtained from treatments T_2 (305g/m^2) & T_5 (305g/m^2), and T_4 (312g/m^2). But there was no significant difference among the yield obtained from T_2, T_5 and T_4. The relative value in grain yield showed that application of RDNF + extra 50% N fertilizer with three splits at heat stress (T_4) showed increased grain yield to some extend (0.69) over RDNF with three splits under heat stress (0.68) but the increment was not significant.

In Fang 60, recommended dose of nitrogen fertilizer with three splits under normal condition (T_1) produced significantly the highest grain yield (441g/m^2) compared to those obtained from all other nitrogen management treatment. Application of RDNF fertilizer with four splits at heat stress growing condition (T_3) showed significantly the

lowest grain yield (251g/m^2). Whereas treatment T_2 (306g/m^2), T_4 (307g/m^2) and T_5 (286 g/m^2) provided the moderate yield and they did not differ significantly among themselves in production of grain yield. The relative value in grain yield showed that application of RDNF + extra 50% N fertilizer did not influence grain yield over RDNF with three splits under heat stress.

In BAW 1059, grain yield was the highest (406g/m^2) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1). Due to different nitrogen management treatments at heat stress condition grain yield decreased significantly. Among them RDNF when was applied with four splits under heat stress condition (T_3) produced significantly the lowest (272g/m^2) which was followed by those obtained from treatments T_5 (315g/m^2), T_2 (321g/m^2) and T_4 (362g/m^2). Application of RDNF + extra 50% N fertilizer with three splits at heat stress (T_4) increased the grain yield significantly over RDNF with three splits under heat stress (T_2). The relative value in grain yield also indicated that application of RDNF + extra 50% N fertilizer with three splits at heat stress (0.89) showed increased grain yield over RDNF with three splits under heat stress (0.79).

In Sonora, recommended dose of nitrogen fertilizer with three splits under normal condition (T_1) produced significantly the highest grain yield (387g/m^2) compared to those obtained from all other nitrogen management treatment. Application of RDNF fertilizer with four splits at heat stress growing condition (T_3) showed significantly the lowest grain yield (202g/m^2). Whereas treatment T_2 (277g/m^2), T_4 (263g/m^2) and T_5 (258g/m^2) provided the moderate yield and they did not differ significantly among themselves in production of grain yield. The relative value in grain yield showed that

application of RDNF + extra 50% N fertilizer did not influence grain yield over RDNF with three splits under heat stress.

The overall results in grain yield indicated that application of RDNF +50% extra N fertilizer with three splits at heat stress (T_4) showed increased grain yield compared to that obtained from RDNF with three splits under heat stress (T_2) in HT Kanchan and BAW 1059 but the increment was significant only in BAW 1059. The grain yield of heat sensitive Sonora was not influenced by extra N at heat stress condition. Increased grain yield in heat tolerant Kanchan and BAW 1059 with extra N application under heat stress condition might be due to increase in ear number/m^2 and greater grain dry weight per ear. Menzies and Whitworth (2004), Malhi and Kutcher (2004), Badaruddin *et al.* (1999) and CuiPing *et al.* (2007) found increased grain yield due to extra nitrogen application under heat stress condition. Badaruddin *et al.* (1999) also reported that the heat tolerant genotype was generally more responsive to additional inputs than the sensitive one.

Above ground biological yield

Wheat genotypes and different nitrogen management at normal and high temperature growing temperature interacted significantly to influence the above ground biological yield (Table 6.5). In Kanchan, above ground biological yield was significantly the highest (1063g/m^2) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1) compared to all nitrogen management treatments under heat stress condition. Above ground biological yield was significantly the lowest (638g/m^2) when RDNF was applied with four splits under heat stress condition (T_3) which was followed by those obtained from treatments T_4 (869g/m^2), T_5 (810g/m^2) and T2 (809g/m^2). But there was no significant difference among the above ground biological yield obtained from T_2, T_5 and T_4. The relative

value in above ground biological yield showed that application of RDNF + extra 50% N fertilizer in three splits at heat stress (T_4) showed increased above ground biological yield to some extend (0.82) over RDNF with three splits under heat stress (0.76) but the increment was not significant.

In Fang 60, recommended dose of nitrogen fertilizer with three splits under normal condition (T_1) produced significantly the highest above ground biological yield ($1065g/m^2$) compared to those obtained from all other nitrogen management treatment. Application of RDNF fertilizer with four splits at heat stress growing condition (T_3) showed significantly the lowest above ground biological yield ($630g/m^2$). Whereas treatment T_2 ($775g/m^2$), T_4 ($773g/m^2$) and T_5 ($735g/m^2$) provided the moderate above ground biological yield and they did not differ significantly among themselves in producing above ground biological yield. The relative value in above ground biological yield showed that application of RDNF + extra 50% N fertilizer did not influence the above ground biological yield over RDNF with three splits under heat stress.

In BAW 1059, above ground biological yield was the highest ($923g/m^2$) when recommended dose of nitrogen fertilizer was applied with three splits under normal growing temperature (T_1). Due to different nitrogen management treatments at heat stress condition the above ground biological yield decreased significantly. Among them the RDNF applied in four splits under heat stress condition (T_3) resulted in significantly the lowest above ground biological yield ($668g/m^2$) which was followed by those obtained from treatments T_2 ($751g/m^2$), T_5($758g/m^2$) and T_4 ($836g/m^2$). The whole RDNF + extra 50% N fertilizer when applied in equal three splits (T_4) and four splits (T_5) at heat stress the above ground biological yield was found to be increased over three split application of RDNF under heat stress (T_2) but the increment was

Table 6.5. Grain yield and above ground biological yield of different wheat genotypes as influenced by different nitrogen managements at normal and heat stress condition

Genotypes	Nitrogen managements	Grain yield (g/m^2)		Above ground biological yield (g/m^2)	
		Actual	Relative to T_1	Actual	Relative to T_1
Kanchan	T_1	451 a	-	1063 a	-
	T_2	305 df	0.68	809 ce	0.76
	T_3	224 hi	0.50	638 g	0.60
	T_4	312 de	0.69	869 bc	0.82
	T_5	305 df	0.68	810 ce	0.76
Fang 60	T_1	441 a	-	1065 a	-
	T_2	306 df	0.69	775 de	0.73
	T_3	251 gh	0.57	630 g	0.59
	T_4	307 df	0.70	773 de	0.73
	T_5	286 dg	0.65	735 ef	0.69
BAW 1059	T_1	406 b	-	923 b	-
	T_2	321 d	0.79	751 e	0.81
	T_3	272 fg	0.67	668 fg	0.72
	T_4	362 c	0.89	836 cd	0.91
	T_5	315 de	0.78	759 de	0.82
Sonora	T_1	387 bc	-	869 bc	-
	T_2	277 eg	0.72	640 g	0.74
	T_3	202 i	0.52	487 h	0.56
	T_4	263 g	0.68	609 g	0.70
	T_5	258 gh	0.67	620 g	0.71
CV(%)		6.52	-	5.70	-

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

T_1-RDNF with three splits (Normal); T_2-RDNF with three splits (Heat)
T_3-RDNF with four splits (Heat); T_4-RDNF+ 50% with three splits (Heat)
T_5-RDNF+ 50% with four splits (Heat)

significant in treatment T_4. The relative value in above ground biological yield also indicated that application of RDNF + extra 50% N fertilizer with three splits at heat stress (0.91) showed increased above ground biological yield over RDNF with three splits under heat stress (0.81).

In Sonora, RDNF in three splits under normal condition (T_1) produced significantly the highest above ground biological yield (869g/m^2) compared to those obtained from all other nitrogen management treatment. Application of RDNF fertilizer with four splits at heat stress growing condition (T_3) showed significantly the lowest above ground biological yield (487g/m^2). Whereas treatment T_2 (640g/m^2), T_4 (609g/m^2) and T_5 (620g/m^2) provided the moderate above ground biological yield and they did not differ significantly among themselves in producing the above ground biological yield. The relative value in grain yield showed that application of RDNF + extra 50% N fertilizer did not influence above ground biological yield over RDNF with three splits under heat stress. Badaruddin *et al.* (1999) also reported 29% increase in biomass production due to application of extra N fertilizer under heat stress condition. The results indicated that equal three spilt application of RDNF +50% extra N fertilizer under heat stress (T_4) showed increased above ground biological yield compared to that obtained from RDNF with three splits under heat stress (T_2) in Kanchan and BAW 1059 but the increment was significant only in BAW 1059.

The overall results of the present experiment indicate that application of additional 50% nitrogen fertilizer in three equal splits increased grain yield only in sink non-limiting, BAW 1059 through influencing flag leaf longevity and stem reserve utilization.

Experment 5. EVALUATION OF THE QUALITY OF WHEAT SEEDS AS INFLUENCED BY LATE PLANTING HEAT STRESSED ENVIRONMENT

INTRODUCTION

High quality planting seed is a key component of all grain cropping systems. High seed quality is needed to ensure adequate plant populations, with reasonable seeding rates, in a range of field conditions. Seed quality at planting represents the integrated effects of the environment during seed production and the conditions to which the seeds were exposed during harvest, processing and storage. Much variation in seed quality has been attributed to differences in environmental conditions prevailing during the formation, development and maturation of the seed while still on the mother plant (Peacock and Hawkins, 1970; Datta *et al.*, 1972). Unfavourable environmental conditions (temperature, rainfall, relative humidity) during seed development and maturation in the field can reduce seed quality.

In Bangladesh, late planting wheat exposed to terminal heat stress, which coincides with grain development and maturation. Evidence consistently shows that exposure of the parent plant to high temperature affects the quality of seed produced. High temperature following anthesis adversely affects grain development in wheat (Tashiro and Wardlaw, 1990b; Hasan and Ahmed, 2005). High temperature accelerates the initial grain growth rate but shorten the grain growth period (Sofield, 1977; Hasan and Ahmed, 2005). As a consequence, smaller and shrunken grain is produced at high temperature. Other physical properties of the seed including seed dimensions, seed coat and the final appearance of the seed are also affected by high parent growth temperature (Tashiro and Wardlaw, 1990b).

In addition to the effects of high temperature on the physical properties of seed, physiological quality is also strongly influenced. Grain development at elevated temperature can affect membrane integrity and can cause increase in membrane leakage of both electrolytes and macro-molecules during germination which subsequently impair germination and seedling vigor (Girelberg *et al.*, 1984). Grass and Burris (1995a) reported impair germination and decline in seed vigor in wheat reflected in reduced shoot and root dry weight and higher seed conductivity due to high temperature during seed development and maturation.

Respiration is one of the most important metabolic factors necessary for successful seed germination. Impaired respiratory activity has been associated with seed deterioration in several species including soybean (Leopold and Musgrave, 1980), cotton (Woodstock *et al*, 1983) and rye (Tluczkiiewcz, 1980). Madden (1992) showed that high temperature during seed development altered respiration rates in imbibed maize axes. However, so far there is little evidence of changes in seed respiratory metabolism that may result from excessive temperature during seed development and maturity. The effect of heat stress on wheat yield and yield components is well documented. But seed quality was often ignored in these studies. Therefore, the objective of the present study was to examine the effect of high temperature during seed development and maturation on seed quality of wheat.

MATERIALS AND METHODS

Location and duration

The experiment was conducted at Crop Botany Laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during the period from November 2007 to January 2008.

Experimental design and treatment

The experiment was conducted in two factor completely randomized design with three replications.

The treatment factors A and B were-

Factor A: Seeds obtained two growing conditions-

1. Normal growing condition (sowing on November 30)
2. Post anthesis heat stress condition (sowing on December 30)

Factor B : Six wheat genotypes

Kanchan
Fang 60
BAW 1059
BL 1022
Pavon 76
Sonora

Seed density determination

Two gram of healthy seed of each wheat genotype obtained from two growing conditions (Normal and heat) was placed in 25ml measuring cylinder containing 20 ml of water. The volume of water replaced by the seeds was measured observing level of water increased inside the measuring cylinder. As the volume of seed was equal to the volume of water replaced by the seeds, the seed density was measured dividing

the seed weight taken by the volume of water removed by the seeds and expressed as g/cc. The experiment was replicated thrice.

Electrical conductivity test

Four-gram healthy seed of each wheat genotype obtained from two growing conditions (Normal and heat) were placed in a test tube (25 mm x 200mm). The seeds were washed three times with deionized water to remove electrolytes adhering to seed. There after 25 ml of deionized water was added in each tube. The initial conductivity reading was taken with an electric conductivity (EC) meter (Model HI 8083, HANNA instruments, Potugal). The test tubes were covered by aluminum foil. Electrical conductivity of seed leachates was measured after 3, 6, 9, 12, 15, 18, 21 and 24 hours and expressed as µS/cm/g of seed after deducting the initial reading. The experiment was replicated thrice.

Germination and seedling vigor test

Germination of seeds (Healthy) obtained from two growing conditions (Normal and heat) was evaluated on two layers of moist paper towel in plastic box (18 ×12 × 4cm). 100 seeds per plastic box were placed and the experiment was replicated thrice. The plastic boxes were covered with lids and placed in Phytotron (NK SYSTEM BIOTRN, Osaka Nippon Medical & Chemical Instrument Co. Ltd. Tokyo, Japan, Model No. LPH-200-RD) with 16 h photoperiod, a light intensity of 200 uE $m^{-2}s^{-1}$, relative humidity of 90±1(%) and at about 20 °C for 8 days. Water was added to the plastic boxes when necessary. Germination was counted at 24-hour interval and continued up to 5th day (120 h) for determination of final germination percentage (%) and coefficient of germination as Copeland, 1976. A seed was considered germinated as radicle came out and were >2 mm long. Number of normal seedlings was counted at 4 and 8 days after placement of germination according to ISTA. At 8 days after

placement of germination 10 normal seedlings were randomly selected from each plastic box and separated from the seed. Then seedlings were dried at 70^{0}C for 72 h and weight was recorded. The mean seedling dry weight was calculated for each treatment combination.

Determination of seed reserve utilization efficiency

Before placement of seed for germination, the seeds for each experimental unit were thoroughly mixed and moisture percentage was determined gravimetrically using a portion of the seeds, the remaining seeds were used for the experiment. Individual weight of 10 seeds for each experimental unit were taken and were placed sequentially according to the marking on filter paper soaked with water in sterilized petridishes. Then the petridishes were kept in seed germinator (ATTEMPTER, Advantec, Japan) at 20^{0}C. For each treatment combination three batches of petridishes each containing 10 seeds were used. Water was added to the petridishes when necessary. At 5th day after placement for germination, five seedlings from each petridish were sampled. Then shoot, root and remaining seeds were dried separately at 70°C for 72 h and weight were recorded.

Seed reserve utilization efficiency (SRUE) was calculated as Hasan *et al*, 2004 using the following formula-

$$SRUE = \frac{SHW + RTW}{SMLR}$$

Where,

SHW = Shoot dry weight

RTW = Root dry weight

RSW = Remaining seed dry weight

SMLR = Seed material loss as respiration

Emergence test

The experiment was carried out in pots (30 × 25cm) filled with soil. 100 healthy seeds were sown per pot at the depth of 3cm and the experiment was replicated thrice. The pots were placed in the net house. The data regarding the final emergence percentage (%) was recorded after the start of experiment.

Relative performance

The relative performance was calculated as Asana and Williams (1965) by the following formula-

$$\text{Relative performance} = \frac{\text{Variable measured under stress condition}}{\text{Variable measured under normal condition}}$$

Data analysis

The data were analyzed using two factor completely randomized design and treatment means were compared by DMRT.

RESULTS AND DISCUSSION

Results on the influence of post anthesis heat stress on the quality of subsequent seed of different wheat genotypes as measured by seed density, conductivity of seed leachates, germination (%), speed of germination, seed reserve utilization efficiency during germination and seedling emergence (%) have been presented and discussed in this part.

Seed density

Seed density of different wheat genotypes obtained from both normal and post anthesis heat stress condition was determined and the results are shown in Table 7.1. The temperature at which the seeds had developed and the wheat genotypes interacted significantly to influence the seed density. The density of seeds obtained from normal growing condition was the highest in heat tolerant Fang 60 (1.363 g/cc) which was statistically identical with those produced by other HT genotypes, Kanchan (1.337 g/cc), BAW 1059 (1.310 g/cc) and BL 1022 (1.310 g/cc). In this condition, The HS genotypes, Pavon 76 (1.25 g/cc) and Sonora (1.25 g/cc) produced seeds with significantly lower density compared to the HT genotypes.

Due to higher mother plant temperature after anthesis, the density of seed reduced significantly in all wheat genotypes except in Kanchan. But the extent of reduction in seed density was lower in HT genotypes (0 to 8%) than those in HS genotypes (9%). The relative seed density value of different genotypes also showed that the HS genotypes affected more (0.91) than the HT genotypes (0.92 to 1.00) in providing seed density by higher mother plant temperature after anthesis. Lu *et al.* (1993) also found reduced grain density in maize due to high temperature during seed development.

Table 7.1. Influence of post anthesis heat stress on density of subsequent seed of different wheat genotypes

Genotypes	Seed density (g/cc)		
	Normal	Heat	Relative
Kanchan	1.337 a	1.337 a	1.00
Fang 60	1.363 a	1.250 c	0.92
BAW 1059	1.310 ab	1.250 c	0.95
BL 1022	1.310 ab	1.250 c	0.95
Pavon 76	1.250 c	1.133 d	0.91
Sonora	1.250 c	1.133 d	0.91
CV (%)	2.03		-

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

Conductivity of seed leachates

The conductivity of leachates (μS/cm/g seed) of seeds obtained from all treatment combiniations increased with time after start of imbibition but seeds produced at high temperature stress released more electrolytes than those produced at normal condition throughout the period of measurement (Fig. 7.1). Among different wheat genotypes, the increment of electrolyte leakage was greater in HS genotypes than the HT genotypes throughout the whole period of measurement. At 24 hour after start of imbibitions, the seeds of HS genotypes (Pavon 76 and Sonora) produced at high temperature released 26 to 27% more electrolytes than those produced at normal growing condition but it was 10 to 18% for the seeds of HT genotypes.

Grass and Buris (1995a) also found increased conductivity of seed leachates in response to heat stress in wheat. Khan and Laude (1969) in their study on barley showed that exposing plants at seed maturity to very high temperature resulted in thinner seed coats. The increase in the conductivity of seed leachates in response to heat stress indicates a difference in the potential vigour of seed obtained from normal and post anthesis heat stress condition.

Germination and seedling vigour

Standard germination test was conducted on seed lots of different wheat genotypes obtained from two growing conditions (normal and heat) after storing the seed 6 months at 10°C, 45% RH. Germination was counted at 24 hours interval and continued up to 5th days for determination of final germination (%) and coefficient of germination and the results are presented in Table 7.2. Number of normal seedlings was counted at 4 and 8 days after placement of germination according to ISTA. Results on number of normal seedlings and seedling dry weight at 8 days after placement of germination are presented in Table 7.3 and 7.4.

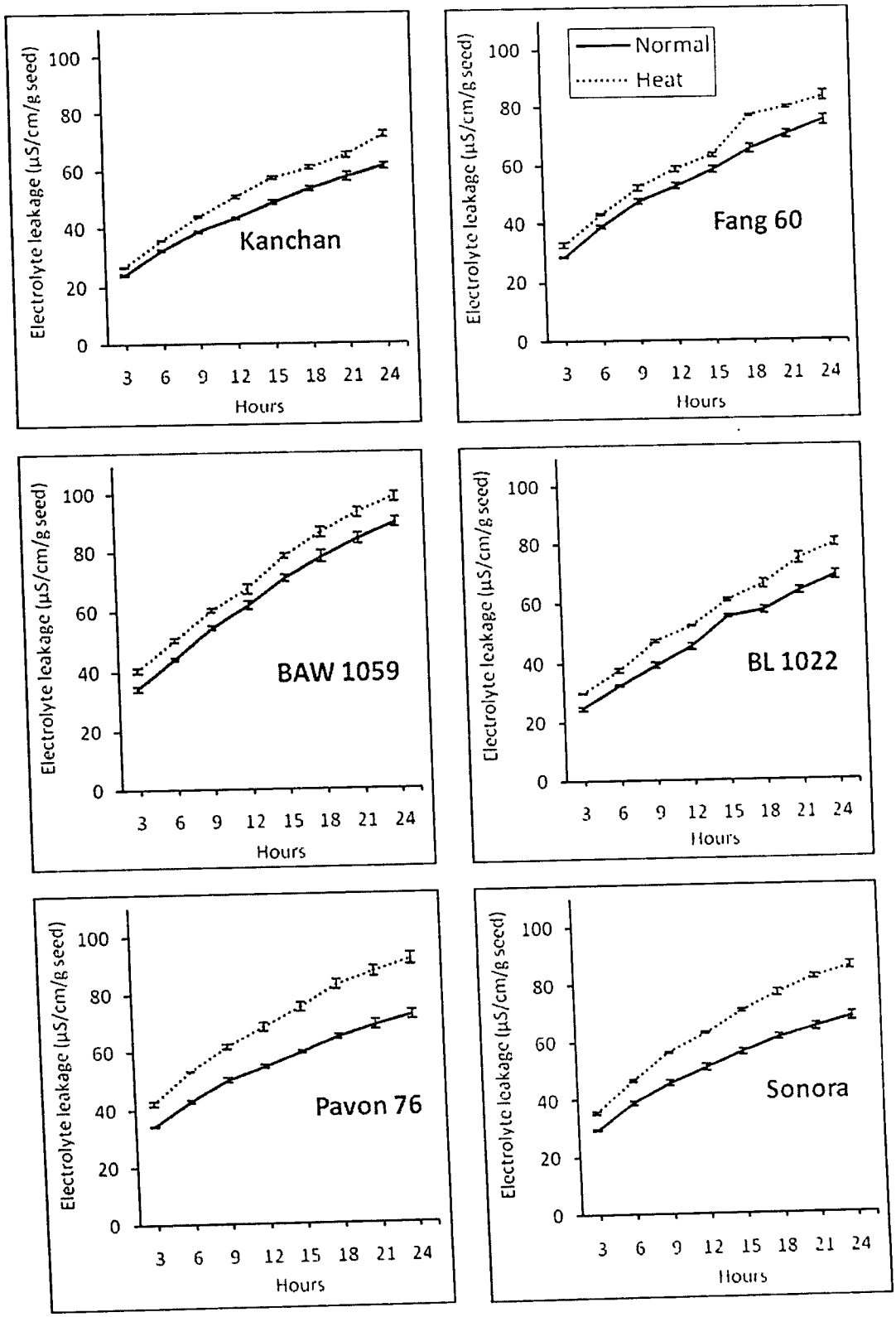


Fig. 7.1. Influence of post anthesis heat stress on the conductivity of leachates (mean ± SE) of subsequent seed of different wheat genotypes at different time after start of imbibition.

Germination (%) and coefficient of germination: Different wheat genotypes and their growing conditions interacted significantly to influence the final germination (%) of the subsequent seed. The temperature at which the seeds had developed and matured did not influence percentage germination for wheat genotypes Kanchan, BL 1022, Pavon 76 and Sonora, whereas seeds of Fang 60 and BAW 1059 obtained from post anthesis heat stress condition showed significantly lower germination (%) than the control.

Coefficient of germination which indicates the speed of germination varied markedly due to the interaction wheat genotypes and their growing conditions. The seeds of Kanchan, Fang 60, BAW 1059, Pavon 76 and Sonora obtained from post anthesis heat stress condition showed significantly lower speed of germination than normal condition, whereas the seeds of BL 1022 showed increased speed of germination though the increment was not significant. Relative value of both germination (%) and coefficient of germination indicates that the change in both of the parameters due to parent plant growing conditions were not distinct between HT and HS genotypes.

Heat stress during seed development and maturation are known to affect seed germination in different plant species (Keigley and Mullen, 1986; Egli *et al.*, 2005 Grass and Burris, 1995a). However, most of these studies were related to seed dormancy. In the present study, to avoid any confounding with seed dormancy, seeds were tested for germination after 6 months storage. The effect of heat stress on parent plants on seed germination after 6 months was not consistent among genotypes. The wheat genotypes, Kanchan, BL 1022, Pavon 76 and Sonora were not affected whereas Fang 60 and BAW 1059 showed lower germination in heat stress treatment during seed development and maturation. Reduced germination could be attributed to seed

damage by high temperature condition during seed development and maturation (Grass and Burris, 1995a). Grass and Burris (1995a) and Moss and Mullett (1982) obtained significant interaction between genotypes and the temperature conditions experienced by the parent plants during seed development and maturation for the wheat seed germination. The same interaction was also significant in the present study. This could be of practical importance to the seed producer in choosing cultivars and the area of production.

Production of normal seedlings: Percentage of normal seedling produced by the seeds was influenced by the combine effect of genotypes and growing condition of their parents both at 4 and 8 days after placement for germination (Table 7.3). Among the seeds obtained from normal growing condition, Kanchan produced the highest number of normal seedlings (96.67%) at 4 days after placement of germination. which was statistically equal to those produced by Fang 60 (95.33%) and Pavon 76 (95.00%). BL 1022 and Sonora produced significantly the lowest number of normal seedlings (89.00%). BAW 1059 (93.33%) produced moderate number of normal seedling which again statistically indentical with those produced from fang 60 and Pavon 76. The seeds of different genotypes obtained from post anthesis heat stress condition produced lower number of normal seedling than the seeds obtained from normal growing condition but the reduction was not significant for Kanchan and BL 1022. Based on degree of reduction, the HS genotypes (Pavon 76 and Sonora) were found to be more affected (13 to 20%) than the HT genotypes (2 to 9%) except the HT genotype Fang 60 which also showed greater reduction (16%) in producing normal seedling at 4 days after placement for germination.

Table 7.2. Influence of post anthesis heat stress on germination (%) and speed of germination of subsequent seed of different wheat genotypes

Genotypes	Germination (%)			Coefficient of germination		
	Normal	Heat	Relative	Normal	Heat	Relative
Kanchan	99.00 a (1.0)	98.00 ac (1.4)	0.99	41.29 de	40.18 f	0.97
Fang 60	99.00 a (1.0)	92.67 d (2.7)	0.94	42.00 c	40.66 ef	0.95
BAW 1059	98.67 ab (1.2)	95.67 cd (2.0)	0.97	43.64 a	42.60 b	0.98
BL 1022	96.33 bc (1.9)	97.33 ac (1.5)	1.01	40.68 ef	41.18 de	1.01
Pavon 76	99.33 a (0.9)	99.00 a (1.0)	0.99	41.65 cd	40.42 f	0.97
Sonora	97.67 ac (1.5)	99.00 a (1.0)	1.01	41.99 bc	40.65 ef	0.97
CV (%)	29.10		-	0.85		-

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.
Values in parentheses are square-root transformation

Table 7.3. Influence of post anthesis heat stress on production of normal seedling (%) from subsequent seed of different wheat genotypes at 4 and 8 days after placement of germination

Genotypes	% Normal seedling at 4 days			% Normal seedling at 8 days		
	Normal	Heat	Relative	Normal	Heat	Relative
Kanchan	96.67 a (1.8)	94.67 ab (2.3)	0.98	95.33 a (2.1)	83.67 cd (4.0)	0.88
Fang 60	95.33 ab (2.1)	80.00 d (4.5)	0.84	94.33 a (2.3)	79.67 de (4.5)	0.84
BAW 1059	93.33 b (2.5)	85.33 c (3.8)	0.91	93.00 ab (2.6)	84.33 cd (3.9)	0.91
BL 1022	89.00 c (3.3)	86.67 c (3.6)	0.97	87.33 c (3.5)	85.67 c (3.7)	0.98
Pavon 76	95.00 ab (2.2)	75.67 d (4.9)	0.80	95.00 a (2.2)	75.67 e (4.9)	0.80
Sonora	89.00 c (3.3)	77.00 d (4.8)	0.87	89.00 bc (3.3)	76.33 e (4.9)	0.86
CV (%)	11.70		-	11.42		-

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.
Values in parentheses are square-root transformation

Among the seeds obtained from normal growing condition, Kanchan produced the highest number of normal seedlings (95.33%) at 8 days after placement of germination. which was statistically equal to those produced by Fang 60 (94.33%), BAW 1059 (93.00%) and Pavon 76 (95.00%). BL 1022 produced significantly the lowest number of normal seedlings (87.33%) which was statistically equal to those produced by Sonora (89.00%). The seeds of different genotypes obtained from post anthesis heat stress condition produced lower number of normal seedling than the seeds obtained from normal growing condition and the reduction was significant except for BL 1022. Based on degree of reduction, the HS genotypes (Pavon 76 and Sonora) were found to be more affected (14 to 20%) than the HT genotypes (2 to 16%).

Reduction in number of normal seedlings produced by the seeds obtained from post anthesis heat stress condition compared to normal growing conditionwas due to increase in number of abnormal seedlings. Grass and Burris (1995a) also obtained greater number of abnormal seedling from the seed obtained from high growth temperature in wheat.

Seedling vigour: Seedling vigour expressed as seedling dry weight was influenced significantly by the combine effect of genotypes and parent plant temperature (Table 7.4). Among the seeds obtained from normal parent plant temperature, Kanchan produced the heaviest seedling (22.60 mg/seedling) which was followed by BAW 1059 (20.57 mg/seedling). Fang 60 produced the lightest seedling (17.27 mg/seedling) which was statistically similar with those produced by BL 1022 (18.37mg/seedling) and Sonora (18.23 mg/seedling). Whereas Pavon 76 produced moderate seedling weight (18.97 mg/seedling) which was statistically identical with those produced by Bl 1022 and Sonora. Seedling dry weight decreased in all wheat genotypes when their

parent experienced heat stress after anthesis and the reduction was significant for all genotypes except BL1022. Based on magnitude of reduction in seedling dry weight, the HS genotypes (Pavon 76 and Sonora) were found to be more affected (23 to 29%) than the HT genotypes (3 to 17%).

Decreased seedling weight with an increase in parent plant temperature was reported by Grass & Burris (1995) and Sechnyak *et al.* (1985) in wheat, by Keigley & Mullen, 1986 and Egli *et al.*, 2005 in soybean, by Fussel and Pearson (1980) in pearl millet grain and by Steiner and Opoku-Boateng (1991) in mature lettuce seed. Most of the previous studies related this decline in vigour to seed size. However, Siddique and Goodwin (1980) showed that seed size alone did not explain the negative effect of high temperature on parent plants. These authors and Moss and Mullett, 1982 suggested that the rate of drying and rapid desiccation affect the maturation of seeds at high temperature might affect the maturation process and attainment of high seed vigour. The results of the present study support that hypothesis, because the seeds developed under high temperature condition matured earlier and dried faster than the seeds produced at normal growing condition.

Seed reserve utilization efficiency

Seed reserve utilization efficiency (SRUE) defined as the ratio of gain in seedling (shoot and radicle) DW to loss in seed DW as respiration during germination was influenced significantly by the combine effect of genotypes and parent plant growth temperature (Table 7.5). Among the seeds of different genotypes obtained from normal parent plant growth temperature, the seed of Pavon 76 showed the significantly highest reserve utilization efficiency during germination (2.075 g/g). The seed of Kanchan showed the second highest reserve utilization efficiency (1.692 g/g)

which was statistically identical with the seed reserve utilization efficiency showed by Fang 60 (1.634 g/g), BL 1022 (1.515 g/g) and Sonora (1.593 g/g). Whereas, the seed of BAW 1059 showed significantly the lowest reserve utilization efficiency (1.245 g/g). Seed reserve utilization efficiency decreased in all wheat genotypes when their parent experienced heat stress after anthesis and the reduction was significant for Kanchan, Fang 60, Pavon 76 and Sonora but not significant for BAW 1059 and BL 1022. Based on magnitude of reduction in seed reserve utilization efficiency, the HS genotypes (Pavon 76 and Sonora) were found to be more affected (32 to 43%) than the HT genotypes (12 to 21%).

Reduced reserve utilization efficiency of seeds obtained from post anthesis heat stress environment might be due to lower seed vigour. Abdul-Baki and Anderson (1973) had reported that any decrease in the metabolic activity will be directly associated with a loss in seed vigour. Grass and Burris (1995b) found that plants that experienced heat stress during their reproductive stage produced seeds with impaired respiratory system. The impairment of respiratory system somehow fails to accumulate the respiratory products in seedling.

Seedling emergence

Seedling emergence (%) was influenced significantly by the combine effect of genotypes and parent plant growth temperature after anthesis (Table 7.6). Among the seeds of different genotypes obtained from normal parent plant growth temperature, Kanchan showed the highest seedling emergence (93.00%) which was statistically similar with all other genotypes except BL 1022 (83.33%). Seedling emergence decreased in all wheat genotypes when their parent experienced heat stress after anthesis and the reduction was significant for all genotypes except Kanchan and BL1022. The relative value showed that the reduction in seedling emergence was

Table 7.4. Influence of post anthesis heat stress on seedling vigour produced from subsequent seed of different wheat genotypes

Genotypes	Seedling dry weight (mg/ seedling)		
	Normal	Heat	Relative
Kanchan	22.60 a	18.87 c	0.83
Fang 60	17.27 d	14.67 e	0.85
BAW 1059	20.57 b	17.40 d	0.85
BL 1022	18.37 cd	17.80 cd	0.97
Pavon 76	18.97 c	13.50 e	0.71
Sonora	18.23 cd	14.00 e	0.77
CV (%)	4.16		-

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

Table 7.5. Influence of post anthesis heat stress on reserve utilization efficiency during germination of subsequent seed of different wheat genotypes

Genotypes	Seed reserve utilization efficiency (g/g)		
	Normal	Heat	Relative
Kanchan	1.692 b	1.331 cf	0.79
Fang 60	1.634 bc	1.293 de	0.79
BAW 1059	1.245 ef	1.028 f	0.82
BL 1022	1.515 be	1.334 cf	0.88
Pavon 76	2.075 a	1.192 ef	0.57
Sonora	1.593 bd	1.077 f	0.68
CV (%)	12.16		-

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

Table 7.6. Influence of post anthesis heat stress on seedling emergence (%) from subsequent seed of different wheat genotypes

Genotypes	Seedling emergence (%)		
	Normal	Heat	Relative
Kanchan	93.00 a (2.6)	90.00 ab (3.1)	0.97
Fang 60	90.33 ab (3.0)	71.67 de (5.3)	0.79
BAW 1059	92.67 a (2.5)	80.00 ce (4.5)	0.86
BL 1022	86.33 bc (3.7)	81.00 cd (4.3)	0.94
Pavon 76	92.00 ab (2.8)	59.67 f (6.3)	0.65
Sonora	91.33 ab (2.9)	70.33 ef (5.5)	0.77
CV (%)	4.56		-

In a column, values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

more (23 to 35%) in HS genotypes (Pavon 76 and Sonora) compared to the HT genotypes (3 to 21%). Lower seed and seedling vigour due to higher parent plant growth temperature after anthesis contributed to the reduced seedling emergence of the seeds of different wheat genotypes obtained post anthesis heat stress condition.

Based on overall results it may be concluded that high parent growth temperature during seed development and maturation resulted in poor seed quality. The effect of parent plant growth temperature on seed germination and speed of germination was inconsistent between heat tolerant and heat sensitive wheat genotypes. Seed vigour as indicated by seed density, seed conductivity, seedling dry weight, production of normal seedling, seed reserve utilization efficiency and seedling emergence was affected more in heat sensitive wheat genotypes than the heat tolerant genotypes due to higher parent plant growth temperature.

SUMMARY AND CONCLUSIONS

Wheat is an important cereal crop and ranks first globally and second in Bangladesh in terms of production and acreage. In Bangladesh, about 60% of the wheat is cultivated at late sowing condition after harvesting the transplanted aman rice. Late planting wheat exposed to high temperature (>26°C mean air temperature) at reproductive stage causing reduction in yield and it is one of the major reasons of yield gap (2 t ha^{-1}). However, this problem will be further increased due to global warming. In spite of low yield of wheat due to post anthesis heat stress, cultivation of wheat can not be avoided totally. Because the irrigation dependent Boro rice cultivation may need to be replaced in future by partially irrigated or non irrigated wheat cultivation to overcome arsenic problem. Therefore, effort ought to be made to minimize the late sown yield reduction by screening or developing high temperature tolerant wheat genotypes/ varieties or by ameliorating the effect of heat stress through agronomic strategies. In order to achieve the objectives three laboratory and two field experiments were carried out during July 2006 to October 2008.

To investigate seed reserve utilization efficiency during germination as screening criteria against heat stress twenty wheat genotypes were tested in the first experiment. The seedling of twenty wheat genotypes were grown on a floating styrofoam stage in water bath for 10 days to measure membrane injury (%) to high temperature. Seeds of those twenty wheat genotypes were also germinated in dark at 25°C and 35°C for 5 days in germinator to determine seed reserve utilization efficiency. Membrane injury (%) of 10 days old seedlings varied significantly among 20 wheat genotypes, with a range of 35.2 to 67.9% and an average of 46.90%. Out of twenty, nine genotypes such as Shatabdi, Prodip, Bijoy, BAW 1064, Sufi, Gourab, Pavon 76, Sonora and

Kalyansona showed less than 50% membrane injury and were classified as HS genotypes. The other eleven wheat genotypes i.e., Kanchan, Fang 60, BAW 1059, BL 1883, BL 1022, IVT 6, IVT 7, IVT 8, IVT 9, IVT 10 and BAW 917 produced equal or greater than 50% membrane injury and were treated as heat tolerant (HT) genotypes. At 25°C temperature, all wheat genotypes showed significantly higher seed reserve utilization efficiency (SRUE) during germination (a mean of 1.549 g/g) as compared to that at 35°C (a mean of 0.880 g/g). The HT wheat genotypes maintained a higher SRUE (1.042 g/g) than the HS genotypes (0.682 g/g) at high temperature stress though both groups of the wheat genotypes maintained a more or less same SRUE (1.531 g/g in HS and 1.563 in HT genotypes) under normal germination temperature. The reduction in SRUE at high temperature compared to 25°C was higher in HS wheat genotypes than in HT genotypes. A significant negative correlation was found across the wheat genotypes between membrane injury (%) and SRUE at 35°C (r = -0.78**).

In second experiment, seedling of 20 wheat genotypes used in the experiment-1 were grown in Phytotron at about 25 °C and 35 °C for measuring membrane injury (%) and also for determination of seedling proline content to investigate seedling proline as screening criteria against heat stress. The wheat genotypes which showed equal or greater than 50% membrane injury were- Shatabdi, Prodip, BAW 1064, Gourab, Pavon 76, Sonora, Kalyansona and IVT 6 which were considered as heat sensitive (HS) genotypes. The other twelve wheat genotypes i. e., Bijoy, Sufi, Kanchan, Fang 60, BAW 1059, BL 1883, BL 1022, IVT 7, IVT 8, IVT 9, IVT 10 and BAW 917 showed less than 50% membrane injury in membrane thermostability test and were grouped as heat tolerant (HT) genotypes. Seedling proline content at 35°C (an average of 1.786 µmoles/g FW) was higher compared to that at 25°C (an average of

0.891μmoles/g FW). At 35°C the HT genotypes produced more than double (>200%) proline than that at 25°C. On the other hand, the HS genotypes produced less than double (<200%) proline at 35°C compared to that at 25°C. The seedling proline content at 35°C and membrane injury (%) maintained a significant negative correlation (r = -0.619**) across the twenty wheat genotypes tested.

Four heat tolerant (Kanchan, Fang 60, BAW 1059 and BL 1022) and two heat sensitive (Pavon 76 and Sonora) wheat genotypes selected from experiment 1 and 2 based on membrane injury (%), seed reserve utilization efficiency and seedling proline level were grown in field under two growing conditions- normal growing condition (sowing on November 30) and post anthesis heat stress condition (sowing on December 30) to evaluate the physiological sensitivity of wheat resulting reduced grain yield in heat stressed environments. Four heat tolerant wheat genotypes (Kanchan, Fang 60, BAW 1059 and BL 1022) showed less than 50% membrane leakage and two heat sensitive genotypes showed more than 50% membrane leakage of flag leaf tissue at anthesis. Distinct changes in kernel proline level between HT and HS wheat genotypes was found at early reproductive phase and in flag leaf, it was found at later reproductive growth stage due to heat stress. Heat tolerance of different wheat genotypes expressed as membrane injury (%) was found to be well associated with heat tolerance in term of kernel proline content at 8 DAA (r = -0.853*) & at 16 DAA (r = -0.860*). This association was also well correlated with heat tolerance in terms of flag leaf proline content at 16 (r = -0.877*) and 24 (r = -0.843*) days after anthesis. Flag leaf longevity reduced in all wheat genotypes due to late planting heat stress but the reduction was inconsistent between HT and HS wheat genotypes. The heat tolerant wheat genotypes showed greater ear and grain growth duration, longer duration of rapid grain growth rate, higher stem reserve utilization and consequently

maintained lesser reduction in grin dry weight per ear and grain yield under heat stress condition than those showed by heat sensitive wheat genotypes. Flag leaf & kernel soluble sugar and flag leaf starch level revealed that the Kanchan, Fang 60, Pavon 76 & Sonora were sink limiting for kernel development under heat stress condition but the limitation appeared 4 to 8 days earlier in HS genotypes than the HT genotypes. The other two HT genotypes, BAW 1059 and BL 1022 were not sink limiting for kernel development and their sink remained active for longer time under heat stress condition. Based on heat susceptibility index, Kanchan (0.83), Fang 60 (0.93) BAW 1059 (0.98) and BL 1022 (0.70) were found as heat tolerant genotypes. On the other hand, Pavon 76 (1.42) and Sonora (1.17) were identified as heat susceptible genotypes.

To evaluate the influence of application of extra nitrogen and/or splitting of nitrogen fertilizer after anthesis to sustain wheat yield due to late planting heat stress two sink limiting heat tolerant (Kanchan and Fang 60), one sink active heat tolerant (BAW 1059) and one heat sensitive (Sonora) wheat genotypes selected from experiment -3 were tested in the field. Application of additional 50% N either as three or four splits increased the flag leaf N in all wheat genotypes but three splits (T_4) was found as more effective than four splits (T_5). Application of additional 50% nitrogen fertilizer under heat stress condition as three splits increased the flag leaf longevity for 2 days in Kanchan and 4 days in BAW 1059 and influenced the stem reserve utilization in BAW 1059 compared to that obtained from RDNF with three splits under heat stress (T_2). Application of RDNF +50% extra N fertilizer with three splits at heat stress (T_4) showed increased grain yield compared to that obtained from RDNF with three splits under heat stress (T_2) only in HT BAW 1059 the sink of which remain active for longer time under heat stress. Increased grain yield in BAW 1059 with extra N

application under heat stress condition was due to increase in ear number/m^2 and greater grain dry weight per ear.

To examine the effect of high temperature during seed development and maturation on seed quality of wheat the seeds obtained normal and high temperature growing condition from experiment-3 were tested for quality. High parent plant growth temperature during seed development and maturation resulted in poor seed quality. The effect of parent plant growth temperature on seed germination and speed of germination was inconsistent between heat tolerant and heat sensitive wheat genotypes. Seed vigour as indicated by seed density, seed conductivity, seedling dry weight, production of normal seedling, seed reserve utilization efficiency and seedling emergence reduced in all wheat genotypes due to higher parent plant growth temperature but heat sensitive wheat genotypes was affected more than the heat tolerant genotypes.

From the overall results it may be concluded that-

- Heat tolerance of wheat genotypes was tested using three methods i.e. cell membrane thermostability test, seed reserve utilization efficiency and seedling proline level in this study. Among these methods seed reserve utilization efficiency was the quickest and a large number of genotypes can be accommodated at a time in this method.
- Estimation of flag leaf proline at rapid grain growth stage was found to be more effective to assess heat tolerance in wheat.
- Heat tolerant wheat genotypes maintained higher stem reserve utilization as well as longer ear and grain growth duration to contribute higher grain dry weight per ear under heat stressed condition.

- The HT Kanchan & Fang 60 and the HS, Pavon 76 & Sonora were found as sink limiting for kernel development under heat stress condition. But the other two HT genotypes, BAW 1059 and BL 1022 were found to have longer sink activity for kernel development due to heat stress.

- Additional 50% nitrogen fertilizer in three equal splits increased grain yield through influencing flag leaf longevity and stem reserve utilization only in genotype, BAW 1059 in which sink remained active for longer time under heat stress condition.

- High parent plant growth temperature during seed development and maturation adversely affected seed quality which was less pronounced in HT wheat genotypes.

RECOMMENDATION

- The heat tolerance in terms of seed reserve utilization efficiency during germination and increased proline level in seedling due to heat stress can be used as physiological screening criteria against heat stress, both of which are equally comparable to cell membrane thermostability test.

- In heat tolerant wheat genotypes, mobilization of proline between kernel and flag leaf should be studied intensively.

- Genotype having longer sink activity under late planting condition, like BAW 1059 was found to be potential for advanced yield trial to sustain wheat yield and also for genetic strengthening of heat tolerance.

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APPENDICES

Appendix 1. Physical properties of soil (0-15cm) of the experimental plot

Soil properties	Observed value
Sand	7.2%
Silt	47.2%
Clay	35.6%
Textural class	Silty clay loam
Bulk density	1.4 g cm^{-3}
Particle density	2.63 g cm^{-3}

Appendix 2. Chemical properties of soil (0-15cm) of the experimental plot

Soil properties	Observed value
Soil pH (Soil: water = 1: 2.5)	5.8
Total N (%)	0.076
Organic C (%)	0.65
Available P (ppm)	18.00
Exchangeable K (meq/100g)	0.46
Exchangeable Ca(meq/100g)	8.75
Exchangeable Mg (meq/100g)	2.46
CEC (meq/100g)	20.06
Sulfur (ppm)	9.25
Zinc (ppm)	1.72
Boron (ppm)	0.74

Appendix 3. Meteorological conditions during crop growing period (2006-2007)

Month	Duration (days)	*Temperature (^{0}C)		*Relative humidity (%)	Total rain fall (mm)	*Evaporation (mm)
		Max.	Min.			
December 2006	1-10	28.0	13.9	85.1	Nil	1.86
	11-20	27.1	15.0	84.3	Nil	1.78
	21-rest	27.1	14.1	84.6	Nil	1.74
January 2007	1-10	22.1	11.0	87.6	Nil	1.10
	11-20	25.5	16.5	62.8	Nil	2.0
	21-rest	27.1	13.0	72.2	Nil	2.1
February 2007	1-10	26.4	17.0	86.8	56.8	2.68
	11-20	26.0	14.8	71.9	11.2	2.52
	21-rest	30.2	17.9	64.0	6.6	3.62
March 2007	1-10	28.9	15.3	68.4	8.4	3.64
	11-20	30.9	17.2	67.6	Nil	4.56
	21-rest	34.1	20.7	73.2	6.4	4.76
April 2007	1-10	34.3	23.7	73.1	6.6	5.62
	11-20	31.8	22.3	74.2	24.2	4.22
	21-rest	32.9	22.3	77.8	58.4	4.74

* Indicated average of the duration (days)
Source: Plant Physiology Division, BRRI, Gazipur.

Appendix 4. Meteorological conditions during crop growing period (2007-2008)

Month	Duration (days)	*Temperature (^{0}C)		*Relative humidity (%)	Total rain fall (mm)	*Evaporation (mm)
		Max.	Min.			
December 2007	1-10	23.0	21.1	84.3	Nil	1.96
	11-20	21.6	18.8	84.0	Nil	1.88
	21-rest	21.5	18.3	84.4	Nil	1.75
January 2008	1-10	21.2	18.3	84.8	Nil	1.74
	11-20	20.0	17.8	84.2	Nil	1.67
	21-rest	18.6	16.7	83.1	4.5	1.54
February 2008	1-10	19.4	16.9	86.1	Nil	1.83
	11-20	20.2	16.0	84.2	55.2	2.61
	21-rest	22.0	19.0	84.6	9.1	2.91
March 2008	1-10	23.3	20.2	84.6	14.3	2.98
	11-20	26.9	24.6	82.9	0.7	3.23
	21-rest	29.4	26.3	80.6	Nil	3.25
April 2008	1-10	30.4	27.1	75.9	10.4	3.71
	11-20	30.4	27.7	77.1	Nil	4.06
	21-rest	31.4	28.5	75.7	Nil	4.11

* Indicated average of the duration (days)
Source: Weather Station, BSMRAU, Gazipur.