

REFERENCE ONLY

PHYSIOLOGICAL CHANGES IN WHEAT UNDER
LATE PLANTING HEAT STRESS

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MD. ABU HASAN

হাজী মোহাম্মদ দানেশ বিজ্ঞান ও প্রযুক্তি
বিদ্যালয়, দিনাজপুর।

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IN
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হাজী মোহাম্মদ দানেশ বিজ্ঞান ও প্রযুক্তি বিশ্ববিদ্যালয়
দিনাজপুর।

BANGABANDHU SHEIKH MUJIBUR RAHMAN AGRICULTURAL UNIVERSITY
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WINTER, 2002

ইত্তা করা হয় না

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MD. ABU HASAN

Registration No. 2001-05-951

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দিনাজপুর।

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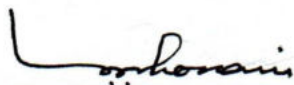
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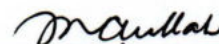
Prof. Dr. Jalal Uddin Ahmed
Major Professor and
Chairman
Advisory Committee



Prof. Dr. Tofazzal Hossain
Member
Advisory Committee



Prof. Dr. M. Mofazzal Hossain
Member
Advisory Committee



Md. Ahsan Ullah
Member
Advisory Committee

**DEDICATED
TO
MY BELOVED SON
'RUMMAN'**

THESIS ABSTRACT

PHYSIOLOGICAL CHANGES IN WHEAT UNDER LATE PLANTING HEAT STRESS

BY

MD. ABU HASAN

In laboratory experiment six wheat genotypes (Aghrani, Kanchan, CB-30, Sonora, CB-24 and CB-34) were germinated to study seed metabolic activity and reserve translocation at 15, 25 and 35⁰C. Among the genotypes four genotypes e.g., Aghrani, Kanchan, CB-30 and Sonora were tested under normal and post anthesis heat stress condition by seeding at November 30 and December 30 to evaluate the physiological changes in relation to heat stress. Speed of germination was influenced by temperature. Seed reserve translocation and seedling growth of Sonora, CB-24 and CB-34 were found to be more affected by the change of temperature than Aghrani, Kanchan and CB-30. Although Sonora, CB-24 and CB-34 showed higher seed metabolic efficiency than rest of the genotypes at 25⁰C, the efficiency of these genotypes reduced more both at 15⁰C and 35⁰C as compared to Aghrani, Kanchan and CB-30. Time to exceed 50% membrane leakage was about four times longer in Aghrani, Kanchan and CB-30 (120-150 min.) and the genotypes were grouped as heat tolerant (HT) than in Sonora (30 min) which considered as heat sensitive (HS). Due to post anthesis heat stress condition the HS genotype Sonora in comparison to heat tolerant (HT) genotype exhibited larger decrease

in kernel proline (47.2%) along with larger increase in flag leaf proline (122%). Irrespective of growing conditions, both dry matter and starch accumulation in kernel showed a typical sigmoidal pattern in all genotypes. Due to post anthesis heat stress, the quantity of dry matter accumulation at peak and duration required to attain peak was higher in HT genotypes than the sensitive one. Sonora, a HS genotype, responded to post anthesis heat stress by accumulating larger quantity of soluble sugar during active kernel development period in contrast to HT genotypes indicating poor capacity of kernel of Sonora to utilize sugar for starch synthesis. The heat tolerant genotypes exhibited distinctly higher relative individual kernel dry weight, higher kernel number per spike, higher relative grain yield and low susceptibility index for grain yield under post anthesis heat stress condition compared to heat sensitive genotype. Finally, grain yield was found to be reduced by about 2.6 to 5.8% in HT genotypes and 7.2% in HS genotype for each 1⁰C rise in average mean air temperature from normal growing condition during anthesis to maturity.

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হাজী মোতাম্মত দারুল বিজ্ঞান ও গুরুত্ব
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CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important cereal crop and ranks first globally and second in Bangladesh both in terms of production and acreage (Anonymous, 1999). Presently the crop covers an area of about 0.85 million hectares and produces about 1.9 million tons in Bangladesh (Anonymous, 2000). But the average yield of wheat is low (2.5 tons/ha) compared to that of many other wheat growing countries of the world as well as the genetic yield potential under Bangladesh condition (Anonymous, 2000).

Late planting of wheat is one of the major reasons of yield reduction because about 60% of the wheat crop is cultivated at late sowing condition after harvesting the transplanted aman rice (Badaruddin *et al.*, 1994). In Bangladesh, late planting wheat exposed to high temperature at reproductive stage causing reduced kernel number per spike (Islam *et al.*, 1993; He and Rajaram, 1993; Al- Khatib and Paulsen, 1990 and Bhatta *et al.*, 1994) and reduced kernel size (Acevedo *et al.*, 1991 and Asana and Saini, 1962). The net effect of these is the reduced grain yield. However, this problem will be further increased due to global warming. Because in Bangladesh also the annual mean temperature of 25.75°C will rise by about 0.21°C and 0.39°C by 2050 and 2100, respectively (Karmakar and Shrestha, 2000).

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.

Identification of wheat genotypes or crop characters of wheat suitable for heat stress condition would be an important step for achieving high yield potential of wheat. In Bangladesh, although some varieties were identified for late sowing condition (Islam *et al.*, 1993 and Ahmed *et al.*, 1975) their heat tolerance is still obscured under Bangladesh condition.⁶ However, the heat tolerance of wheat genotypes can be determined through empirical (yield performance) and/or analytical (physiological trait) methods.

⁶Membrane thermostability (MT) test is a widely used and exceptable method to evaluate heat tolerance and heat susceptibility index is used to evaluate yield parameters. The seed metabolic activity may vary in different temperature regimes (Penning de Vires *et al.*, 1979) and the magnitude of variation was different in different genotypes (Gangadhar Rao and Sinha, 1993). Under supra optimal temperature, free proline content is known to accumulate in different crops (Maiti *et al.*, 2000; Chaitanya *et al.*, 2001; Takeda *et al.*, 1999 and Hossain *et al.*, 1995). Proline is therefore, considered to be a useful component for evaluating the tolerance of a crop to high temperature (Kuo *et al.*, 1968).⁶ But in wheat, the heat tolerance in relation to proline content and seed metabolic efficiency were not yet evaluated not only in Bangladesh but also probably in the other countries of the world.

⁷Post anthesis heat stress in wheat induces several physiological disorder which eventually results in smaller kernel size due to reduced grain filling period (Ishag and

Mohamed, 1996; Stone and Nicholas, 1988) and reduced grain filling rate (Sofield *et al.*, 1977; Tashiro and Wardlaw, 1990) or the combined effect of both (Tashiro and Wardlaw, 1989). However, the physiological and biochemical mechanisms involved in temperature dependent grain growth are yet to be studied. Finally, to elucidate heat tolerance and associated physiological changes in wheat the present investigation was carried out with the following objectives-

1. To determine the heat tolerance in wheat genotypes by using seed metabolic activity for germination, thermostability test for electrolyte leakage through membrane and heat susceptibility index for grain yield.
2. To investigate some physiological changes in grain involved in heat tolerance under variable temperature regimes.

CHAPTER II

REVIEW OF LITERATURE

Heat tolerance mechanism of wheat is to be investigated for developing heat tolerant genotypes for the tropics. Studies on the physiological aspects of heat tolerance and screening of heat tolerant wheat genotypes are important components for it. A very little work has been done on heat tolerance of wheat in Bangladesh. But numerous works have been done in this area in different wheat growing countries. However, information available in respect of heat tolerance of wheat pertinent to present investigation are presented in this chapter.

2.1. Germination and seed metabolic activity as influenced by temperature

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).

Sultana *et al.* (2000) studied the effect of various constant temperatures (4, 9, 14, 19, 24, 29, 34 and 38⁰C) on the germination of winter wheat seed in the dark. The maximum germination percentage was 98 at 24⁰C. The speed of germination was faster at 29⁰C. The most suitable temperature for germination was in the range of 24 to 29⁰C. Alpha amylase expression during germination was also high at higher temperature and maximum expression occurred at 29⁰C, although a high temperature of 38⁰C prevented the synthesis of alpha-amylase. The close correlation between germination and alpha-amylase activity at various temperatures indicated that alpha-amylase was an essential

factor for the temperature dependent germination of wheat seed. Gimalov *et al.* (1996) also reported that the rate of synthesis of some proteins depended on germination temperature.

Germination temperature influenced the root and shoot growth and dry matter partitioning. Gangadhar Rao and Sinha (1993) conducted an experiment with two sorghum hybrids and their parents at 20°C, 30°C and 40°C to find out the efficiency of seed reserve utilization during seed germination. They found at low temperature the proportion of dry matter in shoot and root was almost similar, but at high temperature, dry matter accumulation was more in shoot than in roots. The seed metabolic efficiency showed substantial improvement in all genotypes at higher temperature of 30°C. At 40°C, there was not much improvement in the seed metabolic efficiency.

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). According to Penning de Vries *et al.* (1979), above a temperature of 25°C in wheat or 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.

2.2. Membrane thermostability and heat tolerance

Cellular thermotolerance in terms of cellular membrane thermostability (MT) is often implied as an indication of crop plant heat tolerance and it is therefore considered as a possible selection criterion for heat tolerance (Blum *et al.*, 2001). They also reported that the association between MT and yield under heat stress were reasonably strong and

significant but not perfect in 49 breeding lines of wheat. Other factors besides MT may support yield under heat stress.

Comparison of heat tolerance for eight wheat cultivars based on yield levels and the cell membrane thermostability technique showed that the most tolerant and most susceptible cultivars were given the same ranking by the two techniques, while results varied for the intermediate cultivars (Sharma and Tandon, 1998). Fokar *et al.* (1998) also reported positive significant correlation between membrane thermostability and yield of 16 spring wheat cultivars in four hot environments in Mexico, Sudan, India and Brazil.

A cell membrane system that remains functional during heat stress finally contributes to adaptation of plants to high temperature (Raison *et al.*, 1980). This is especially important during the grain filling period, since grain growth is greatly reduced by environmental conditions that adversely affect assimilate supply (Evans *et al.*, 1975) or utilization (Bhullar and Jenner, 1985, 1986).

Sullivan and Ross (1979) measured the amount of electrolyte leakage from leaf discs bathed in deionized water after exposure to a heat shock treatment. They interpreted the measurement as an indicator of membrane thermostability (MT) in response to heat stress. They used this MT procedure to identify genetic variation in heat tolerance in grain Sorghum. Genetic variation determined by this procedure was related to differences in whole plant photosynthesis under high temperatures, as well as field performance of several cultivars grown under high temperature stress. Raynolds *et al.* (1994) also reported the membrane thermostability showed promise as predictor of heat resistance.

The electrolyte leakage or membrane thermostability (MT) test was conducted by Shanahan *et al.* (1990) at anthesis on flag leaves of eight fields grown genotype of spring

wheat (*Triticum aestivum* L.). Based on MT values, genotypes were grouped as heat tolerant (HT, n=4) Vs. heat sensitive (HS, n=4). The HT and HS genotypes produced similar grain yields at the northern and central locations. However, the heat tolerant group of genotypes produced 21% more grain yield than the HS group at southern site, which was considerably warmer during the grain filling period than the other two locations. Average temperature during July were 19.5, 21.7 and 23.4⁰C at the northern, central and southern locations respectively. These results suggest that the greater MT of HT genotypes was associated with productivity only under extreme temperature. Based on results of this study it seems that MT test is a useful screening procedure for selecting HT genotypes of spring wheat.

Saadalla *et al.* (1990) determined the relationship between membrane thermostability (MT) and other agronomic traits of winter wheat (*Triticum aestivum* L.). A trial of 144 genotypes was evaluated for MT test and the results were expressed as relative injury (RI). Among the genotypes the relative injury varied significantly with a range of 31 to 78%. Based on RI values, the genotypes were separated into three groups: the heat tolerant (HT), Intermediate tolerant (IT) and heat sensitive (HS). Accordingly, 27 genotypes were classified as HT, 71 as IT and 46 as HS. Yield, grain volume per weight, and kernel weight were greater for HT than IT or HS genotypes. Thus, heat tolerance as evaluated by the MT test, was associated with grain yield and quality. These results suggest that the MT test would be a suitable procedure for selecting heat tolerant winter wheat genotype in a breeding program.

Hossain *et al.* (1995) developed an improved method of determination of membrane thermostability (MT) for screening heat tolerance and sensitive varieties in

Brassica. Among four varieties of cabbage (*Brassica oleracea* var. *capitata* L.) studied, the varieties 'Yoshin' and 'YR kinshun' were the most tolerant followed by 'Sousyu' while the variety 'Kinkei no. 201' was heat sensitive. The heat killing time of heat tolerant and sensitive varieties was 150 and 120 min. at the elevated temperature of 55°C, respectively. In Chinese cabbage (*B. campestris* var. *pekinesis* L.) the varieties 'Kenshin' and 'No. 11' were heat tolerant, whereas the variety 'Chihiri 70' was heat sensitive. In Chinese kale (*B. oleracea* var. *alboglabra* Bailey), the variety 'Full white' and 'Sen-yoshirobana' were heat tolerant while the variety 'large leaf' was heat sensitive. In general, heat tolerance ability was higher in Chinese kale compared to Cabbage and Chinese cabbage. The heat tolerance trait in the interspecific somatic and sexual hybrids between Cabbage X Chinese cabbage and between Chinese kale X Chinese cabbage was intermediate between that of the parents. So, for screening and analyzing the genetic variability of heat tolerant variety the MT test is efficiently used.

Heat tolerant and susceptible genotypes did not differ in heat killing time when the plants were grown at 20/15°C day/night temperature. However, the differences became dramatic after the plants were acclimated at temperature above 30°C for 24 hours (Chen *et al.*, 1982). Heat killing time was a function of the acclimating temperature with the optimum acclimating temperature found 35 to 37°C. Rate of heat acclimating was very fast, and a continuous high temperature treatment appeared to be necessary to retain the level of heat tolerance. They also suggested that the heat hardiness of crop plants could be detected by measuring the ion leakage immediately after a stress of high temperature, i. e. 50°C. Leaves could serve as the testing tissue for screening heat tolerant genotypes with plants as young as 1-month old.

Balota and Saulescu (1994) compared seedlings of seven wheat genotypes using a modified thermostability test and observed that wheat genotypes ranking for MT corresponded well with the ranking based on previous field observations on grain filling at high temperatures.

Based on membrane thermostability test Sikder *et al.* (1999) classified wheat varieties (Ananda, Pavon, Aghrani and Barkat) which took maximum heat killing time as relatively tolerant, three varieties (Akbar, Kanchan and Protiva) as moderately heat tolerant and three varieties (Balaka, Sawgat and Sonora) which took the lowest heat killing time considered as heat sensitive.

2.3. Proline content and heat tolerance

Free proline is accumulated in plants in response to a wide range of biotic and abiotic stress such as water stress, salinity, temperature, pathogen infection, pollution and nutrient deficiency/toxicity (Hare and Cress, 1997). Maiti *et al.* (2000) reviewed the accumulation of proline for osmotic adjustment is a mechanism that resistant plants have to adapt to abiotic stress factors such as salinity, high temperature and drought in various crop species.

Chaitanya *et al.* (2001) found that proline accumulation was 1.5 fold 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 heat stress there were greater differences in proline accumulation among 6 cotton cultivars was obtained by Ronde *et al.* (2001). Tokeda *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 the effect of high temperature on proline content in tomato floral buds and leaves. The results indicated 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.

Hossain *et al.* (1995) determined the proline content in different cultivars of Cabbage, Chinese cabbage and their hybrids under high temperature stress to identify differences in heat tolerance. Proline content of the leaves of inter-specific hybrids between Cabbage cultivar (c.v.) 'Yoshin' and Chinese cabbage (c.v.) Kenshin (*B. campestris* var. *pekinensis* L.) and between Chinese Kale c.v. Sen-yo-shirobana (*B. oleracea* var. *alboglabra* Bailey) and Kenshin was intermediate between those of their parents at 35°C. The proline content of stalks and floral buds at 25°C and 35°C were found higher than leaves. There had a negative correlation between pollen viability and the rate of decrease of the proline content in the floral buds. It had been observed that the

increase in proline content was 1.2, 1.6 and 1.8 times in the leaves of heat tolerant Cabbage c.v. 'Yoshin' and Chinese cabbage c.v. 'Kenshin' and Chinese Kale c.v. Full White respectively. In contrast, the increase in the proline content of the heat sensitive cabbage c.v. YR Kinshun, Chinese cabbage c.v. 'Chihiri 70' and Chinese Kale c.v. 'Large leaf' were 3.5, 17.6 and 3.6 folds respectively. They finally suggested that proline accumulation may be used as a criterion for selection of heat tolerant genotypes in Brassica.

Lalk *et al.* (1985) drew conclusion that accumulation of proline in plants during various environmental stresses happened to occur which was subsequently supported by Machakova *et al.* (1989) and used as an index of stress resistance. Adaptation to temperature and water stress is closely associated with proline accumulation was uttered by Handa *et al.* (1986) and Watad *et al.* (1983).

Bogges *et al.* (1976) stated that the synthesis of many metabolites is closely controlled by end-product inhibition. The synthesis of proline in maize roots (Oaks *et al.*, 1971) and tobacco leaves (Noguchi *et al.*, 1968) had been suggested to be controlled similarly. First, end product inhibition may not occur at all in plants which accumulate proline in response to stress; rather, proline levels in these species may be controlled by rates of proline utilization in protein synthesis and proline oxidation. On the other hand, end-product inhibition of proline biosynthesis may operate in turgid plants, but fail under stress. That is the critical effect of stress in proline accumulating plants might be the disruption of normal feedback inhibition of proline biosynthesis.

Proline accumulates in the seedlings as well as cultured cells of tomato because of short-term heat shock and cold shock in relation to calcium involvement was cited by

Bhattacharjee and Mukherjee (1996). Park *et al.* (2001) measured leaf proline content as a water and high temperature stress indication in apple. They found that proline content was high in both water and high temperature stress and there was a significant interaction between root zone temperature and osmotic potential for proline and water content of leaves.

Wojciechowska *et al.* (2001) 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.

Talwar *et al.* (1999) estimated different amino acids in three groundnut cultivars ICG 1236, ICGS 44 and Chico grown under day/night temperature regimes of 25/35 and 35/30⁰C. They found that high temperature resulted in the accumulation of amino acids (proline, glutamine, serine, amino butyric acid and arginine) in leaves of all cultivars, but in floral buds of ICG 1336 only. Reduction in the concentration of proline in anthers was noticed with the advancing anther development in all the cultivars at both temperatures. Anther of ICG 1236 accumulated about 50 n moles/mg fresh weight more proline at 35/30⁰C than at 25/25⁰C at 5 days before anthesis stage. Although a reduction in the proline concentration in the stigma+style tissue under higher temperature in all the cultivars was evident.

Zhang *et al.* (1998) estimated free proline concentration and catalase activity in five cucumber cultivars differing in heat tolerance subjected to heat stress (36⁰C) for 1, 3 and 5 days at the 3-leaf stage. The results showed that relative increment percentage for

free proline content, particularly after the 3-day treatment was discriminating and coincided completely with the heat tolerance ranking of the cultivars. No significant relationship was observed between heat tolerance and catalase activity.

The proline concentration in citrus leaves differed between species and cultivars in response to temperature. As the maximum air temperature increased during summer months (42-44⁰C) and the minimum air temperature decreased during winter months (around 5-8⁰C) 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). Hare and Cress (1997) reported the contribution of proline to oxidative respiration and involvement of proline metabolism in the regulation of intracellular redox potential and proline as stress related signal.

2.4. Kernel development and its physiology

Under high temperature, the wheat crop completes its cycle much faster than under normal temperature conditions (Fischer, 1985). All crop stages have a short duration, consequently, there are fewer days to accumulate assimilates during the life cycle and production of biomass is reduced (Fischer and Maurer, 1976). Average grain filling duration was shortened by four and maturity by six days in the later planted warmer conditions. Despite these reductions, some genotypes showed stability of grain yield between the two planting periods indicating possible genetic variability in grain filling rate.

Generally reduced kernel weight in heat stressed wheat results from shorter duration rather than reduced rate of grain growth (Sofield *et al.*, 1977; Warrington *et al.*, 1977; Wiegand and Cuellar, 1981). Thus, heat stress during grain-filling hastens maturation of the grain, resulting in smaller, often shriveled grains.

Day/ night temperature of 28/20 °C accelerated grain development compared with that at 23/15 °C, while dry matter accumulation and cell division occurred at a higher rate but had a shorter duration (Nicolas *et al.*, 1984).

Grain filling duration generally has inverse relationship with the prevailing temperature. Heat stress during GS₃ phase (anthesis to maturity) results in faster senescence of foliage, poor assimilate availability, reduced translocation of photosynthates to the developing grain and greater respiratory losses (Al-Khalib & Paulsen, 1984). Consequently, the net effect of heat stress at this stage (GS₃) is lower kernel weight due to reduction in the grain - filling period and grain filling rate or the combined effect of both (Tashiro & Wardlaw, 1989). Based on several studies, Acevedo *et al.*, (1991) concluded that grain weight was reduced from 4 to 8% for each 1°C rise in daily mean temperature.

Sikder *et al.* (1999) found that in optimum sowing (November 30) the tolerant and sensitive wheat varieties had the similar grain filling duration (40 days). However, in December 30 sowing, the tolerant variety had longer grain filling duration (32 days) compared to sensitive variety (28 days). Both the tolerant and moderately tolerant varieties showed lower pre-anthesis stem reserves contribution to the final grain weight compared to November 30 sowing. On the other hand, the heat sensitive varieties had higher pre-anthesis stem reserves contribution at December 30 sowing condition. Sharma

and Tandon (1998) also found that reduction of the duration of the period from anthesis to maturity due to high temperature ranged from 18 to 20% in eight wheat cultivars.

The early maturing wheat genotypes showed better performance under high temperature stress with less reduction in grain yield and had relatively higher grain growth rate with more tolerance to heat stress for most of the yield contributing characters (low heat susceptibility index). Grain growth rate and heat susceptibility index could be taken as important criteria for breeding genotypes acclimatized to late planting conditions (Sarker *et al.* 2001).

Toru and Wardlaw (1988) found a reduction in the grain growth duration and dry matter accumulation rate with increasing temperature above a mean of 26.7°C. Wheat cultivars that can fill their grain quickly may have an advantage in environments with short, hot and dry grain filling periods (Whan *et al.* 1996).

Tashiro and Wardlaw (1989) observed that when wheat cv. Banks was subjected to a range of temperature from 17.7 to 32.7 °C the 1st anthers appeared seven days later, grain weight at maturity was reduced by about 5% for each 1°C rise in temperature within this range and there was a reduction in the duration of grain growth with increasing temperature.

High temperature in the post anthesis period shortens the duration of grain - filling (Wiegand and Cuellar 1981) such that each degree increase during grain filling results in about three days decrease in the duration of filling regardless of cultivar (Asana and Williams 1965, Begga and Rawson 1977). Evans and Wardlaw (1976) and Sofield *et al.* (1977) concluded that neither illumination conditions nor grain filling rates affect the duration of grain filling.

Amores *et al.* (1984) observed that wheat cv. Sonora 64 was transferred to 27°C during 6 development stages and compared with control plants grown continuously at 17 or 27°C. The time from sowing to maturity was reduced more by later exposures (after anthesis to maturity) to high temperature. High temperature during the vegetative phase (sowing to leaf primordia) the transition phase (flag leaf primordia to double ridge) and spikelet initiation resulted in greater setting of fertile florets but a decreased in the number of fertile florets/ear and individual grain weight.

Rawson (1988) examined the hypothesis that rapid phenological development, poor biomass production and sterility are major factors leading to the poor yields in wheat grown from sowing to maturity at high temperatures. Twenty-four wheat genotypes at moderate temperatures were compared in a glasshouse study of 'minicanopies' of plants grown at 30/25°C. Absolute differences among genotypes in growth and floral development become small at this high temperature. However, a short vegetative period did not prevent rapid rates of biomass production and the emergence of at 6 ears per plant. There was little evidence that high temperature resulted in floret sterility except 2 genotypes and consequent harvest index was generally very high. The range in grain yields among genotypes was 0-10 g per plant, >70% of genotypes gave >7 g per plant. It is suggested that high temperature tolerance already exist in many common wheat.

Accelerated development, enhanced respiration and numerous indirect effects complicated interpretation of high temperature injury to plants (Chowdhury and Wardlaw, 1978; Fischer, 1985; Rawson, 1988; Sofield *et al.*, 1977; Spiertz, 1977; Wardlaw *et al.*, 1980; Welbank *et al.*, 1968).

Asana and Williams (1965) carried out an experiment to determine the effects of high temperature on grain development and yield in wheat. Two Australian and three Indian cultivars of wheat were exposed from a week after anthesis until maturity, to day temperatures of 25, 28 and 31°C and night temperature of 9 and 12°C. There was a mean reduction in yield of 16% for 6°C rise in day temperature but the cultivar did not differ significantly in their response to these temperatures. There were no significant effects of night temperature on grain weight but stem weight was less at 12°C. Mean weight per grain provided the best index of treatment effect and there were considerable differences between cultivars for these attributes. Relative effects of treatment were especially meaningful and grain weights per main-shoot ear were affected to the same relative extent by treatment, but were inherently more variable. Senescence was hastened only slightly by high day temperature, and there were no differential effects between cultivars in this respect. Growth analysis of the Australian cultivars Ridley and Diadem indicated the developing grain of Ridley had a greater capacity for growth than that of Diadem from the earliest stage. This together with the confirmation of grain size as a very stable character for all varieties to the developmental and synthetic activity of the grain was an important determinant of grain yield.

High temperature (35° days/ 25°C night) adversely affected the source and sink activities. Heat stress accelerated the decline in viable leaf blade area and photosynthetic activity per unit leaf area. Grain filling rate decreased from the lowest (25°C day/ 15°C night) to the highest temperature (35°/25°C), but the change was smaller than the decrease in grain filling duration at same temperature. A major effect of high temperature appeared to

be acceleration of senescence, including cessation of vegetative and reproductive growth and deterioration of photosynthetic activities (Al-Khatib and Paulsen, 1984).

Jhala and Jadon (1989) studied that grain growth rate (mg/spike per day) from the 1st to the 8th week after anthesis (WAA) in 15 wheat cultivars sown on 15 November (optimum date) or 30 November. There were significant differences among cultivars for grain growth rate especially during 1st 4 WAA. Grain growth rate was the highest in the 3rd and 2nd WAA for crops sown on 15 November and 30 November respectively. Grain growth rate was higher in crops sown on 15 November than in those sown on 30 November. Cv. Lok 1, WH 147, H-I784, Kalyansana and HJ 74-27 had initial higher grain growth rate and could be used for breeding cultivars suitable for late sown conditions.

Wheat variety HD 2428 and Kalyansona were compared by Shukla *et al.* (1992) for adaptability under pot culture by exposure to high temperature treatments (8°C above ambient) in weeks 1 though 4 after anthesis. Dry matter accumulation of grain in the top, middle and bottom spikelets of the spike, at 7-grain locations was recorded in weeks 2 and 3. The treatments adversely affect grain weight for HD2428 at all 3 spikelet positions, with up to 35% reduction in the first 5 grain location. Kalyansona was only marginally affected. This indicates that the characteristic adaptability of Kalyansona to different agroclimatic regions is associated with the tolerance of physiologically old grains to higher temperature.

Wardlaw (1974) found that in wheat, translocation through the stem was not inhibited until temperature fall below 1°C. The short period at 50°C did not appear to reduce translocation, but inhibition occurred, if the 50°C treatment was prolonged. However, a temperature of 40°C did not inhibit translocation even after an exposure of 3

days. Gallagher *et al.* (1975) postulated that maximum possible contribution to grain weight by material assimilated before anthesis was less than 20% although under adverse environmental conditions it may increase up to 74% of the final grain weight. Sadeque (1990) measured 10-18% and Austin *et al.* (1980) measured 11-12% of the final grain weight in Barley translocated from pre-anthesis assimilation under normal environmental conditions. Whereas, Haque (2002) found that photosynthate stem reserve translocation (PSR) from stem to grain varied from 25.05 to 30.72% in heat tolerant wheat genotypes and 19.95 to 25.15% in heat sensitive genotypes under normal growing condition but under late growing condition, PSR was 23.27 to 34.02% in heat tolerant wheat genotypes and 36.12 to 41.05% in heat sensitive genotypes.

Chanda *et al.* (1999) found that the data of dry matter accumulation and starch content were fitted to a sigmoid curve which revealed a close correspondence between these two parameters in three wheat cultivars. Reducing and nonreducing sugars were high during early stage but declined with advances in growth up to almost middle of the grain growth, high levels were even found at later stage. The activity of RuBPC and SPS in leaf extracts in Mulberry plant markedly reduced at high temperature. Increased leaf temperature affected sugar metabolism through reduction in leaf starch content and sucrose starch balance (Chaitanya *et al.* 2001).

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). Lafta and James, (1995) found that heat stress increased accumulation of foliar sucrose and decreased starch in mature leaves but did not affect glucose. SPS activity increased significantly in mature leaves but the activity of sucrose synthase and

ADP-glucose pyrophosphorylase was reduced in tuber of Potato. 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.

Viswanathan *et al.* (2001) analyzed the effect of heat stress on the components of grain weight in wheat cultivars differing in grain weight stability. The wheat cultivars PBS 154, Sonalika and Hindi 62 were grown in the field of three dates of sowing, 19 November 1992, 14 December 1992 and 18 January 1993. The late sown wheat experienced 6-8⁰C warmer temperatures during grain development than crop sown at the normal time. The heat susceptibility index revealed that grain weight was less susceptible to heat in Sonalika and PBW 154 than in Hindi 62. Heat stress reduced both the grain growth duration (GGD) and grain growth rate (GGR). The grain weight reduction in PBW 154 and Sonalika was mainly due to a reduction GGR, while that of Hindi 62 was due to a reduction in GGD. In vivo studies on starch in excised endosperm at 15, 25 and 35⁰C revealed that this was more thermotolerant in Hindi 62 than in PBW 154. The grain starch content was stable in Hindi 62 while that in PBW 154 was significantly reduced under heat stress. It was concluded that the heat susceptibility of grain weight in Hindi 62 was mainly due to a reduction in GGD, although GGR and starch synthesis were more thermotolerant in developing grains of Hindi 62 than in those of PBW 154.

Cruz *et al.* (2001) investigated the duration of grain filling, patterns of partitioning of ¹⁴C photoassimilates between organs and incorporation of the label into starch in the kernels 24h after feeding the flag leaf blade with ¹⁴CO₂ in different sowing

time with contrasting temperature conditions to evaluate source sink balance. They found that the proportion of ^{14}C converted into starch in the kernels increased whereas the allocation to the internodes decreased during the first 10 days of grain filling. The patterns of ^{14}C photo assimilate partitioning differed among sowing dates. Lack of such a relationship at later stages in grain development indicates a change from sink limited to source limited situation early after anthesis. They concluded that the supply of photoassimilates under field conditions in Cuba limits grain filling, except the first 10 days after anthesis when it was sink-limited.

2.5. Yield and yield contributing characters

Hexaploid wheats are of temperate origin (Harlan and Zohary, 1966). The optimum temperature for wheat crop is about 20°C (Al-Khalib and Paulsen, 1984). Like most species of temperate origin, wheat does not tolerate prolonged exposures to temperatures exceeding 35°C (Gusta and Chen, 1987). 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).

Crop growth resource components like leaf area development (LAD) and tillering are determined during GS_1 phase (emergence to double ridge). Sensitivity to heat stress during this phase is expressed as decreased duration of GS_1 , reduced LAD, number of leaves and spike bearing tillers (Shpiler and Blum, 1986). Mean temperature of 16 to 20°C is favorable for tillering. Duration of GS_1 was reduced by about 34d with concomitant loss (82%) of leaf area index and total spike m^{-2} when mean seasonal temperature changed from 12.2 to 27°C (Acevedo *et al.*, 1991). High temperatures accelerate organ production without any increase in net photosynthesis (Begga and Rawson, 1977) resulting in smaller organs.

Bhatta *et al.* (1994) evaluated the variation of twelve spring wheat genotypes of similar maturity in grain yield, morphophysiological characters and the relationship between grain yield and plant traits over two planting periods. Most of the characters were altered when planting was delayed by one month from the normal seeding date. Decline in grain yield, kernels per spikelet and 1000-grain weight were relatively of small magnitude.

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).

Productivity of wheat and other temperate species fall markedly at high temperature (Bhullar and Jenner, 1983; Rawson, 1986; Shpiler and Blum, 1986; Wardlaw *et al.*, 1980). Genotypes within species differ in response to high temperatures, however, indicating substantial genetic variability for the trait. Wheat genotypes for instance, 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 GS₂ phase (double ridge to anthesis). Shpiler and Blum (1986) observed that the cultivars that sustained the highest yield in hot environments were able to maintain the longest duration of GS₂ and had the highest number of grain per spike. 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 GS₂ stage.

Al-Khatib and Paulesn (1990) evaluated the yield performance of 10 wheat genotypes grown under moderate (22/17°C, day/night) and high (32/27°C, day/night) temperature. Yield component of 10 genotypes at maturity reacted differently to high temperature. Spike per plant significantly decreased in 3 genotypes and increased in one genotype as the temperature increased whereas kernel per spike decreased in four genotypes. Kernel weight decreased significantly in all genotypes by a mean of 20% whereas the reduction range was about 10% to 30%. Grain yield means declined from 0.75 to 0.58 g per tiller 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 all 10 genotypes was affected little by temperature, but individual genotypes responded differently.

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).

Kanani and Jadon (1985) assessed 110 wheat genotypes for ability to withstand high temperature when sown early to fit into a rotation with groundnut 18, including Hindi 62, C306, K65, NP846, HJ72-6, HJ72-50, HJ72-65, HI617 and W6357, were superior. These genotypes had higher values for yield and 5 yield related traits when sown early than when sown late, for example, Hindi 62 yielded twice as much with earlier as with late sowing. 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.* (1998) observed significant variation among five wheat cultivars in the reduction in grain weight per ear, kernel number and single kernel weight under heat stress. Differences in grain weight per ear among cultivars were ascribed to the variation in the reduction in both kernel number and kernel weight under heat stress.

Grain yield of wheat decreases and grain protein increases with the increasing temperature stress duration (Ahmad *et al.* 1989).

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/m², 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. Aghrani 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 filling stage. Evans *et al.* (1975) and Mudholkar (1981) reported that higher temperature at grain filling stage was one of the important reasons for lower grain yield in wheat crop.

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 and not genetic factor as previously held.

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.

CHAPTER III

MATERIALS AND METHODS

In the present investigation, two experiments were conducted separately, as

Experiment – I : Influence of temperature regimes on seed reserve mobilization in wheat genotypes

Location and duration

The experiment was conducted at Crop Botany Laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur during 15 October to 15 November, 2001.

Experimental design and treatments

The experiment was carried out in two factors completely randomized design with three replications.

The treatment factors A and B were -

- A. Three germination temperature 15, 25 and 35⁰C
- B. Six wheat genotypes (Aghrani, Kanchan, CB-30, Sonora, CB-24 and CB-34)

Collection of seed

Seeds of six wheat genotypes (Aghrani, Kanchan, CB-30, Sonora, CB-24 and CB-34) were collected from genetic resources unit of Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur.

Seed placement for germination

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 30 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 15, 25 and 35°C. For each temperature three batches of petridishes each containing 30 seeds were used. Water was added to the petridishes when necessary.

Rate of germination, co-efficient of germination and germination vigor

Germination was counted at 24-hour interval and continued upto 5th day (120 h). A seed was considered germinated as plumule and radicle came out and were >2 mm long.

The rate of germination was calculated following Krishnasamy and Seshu (1990), as-

$$\text{Rate of germination (\%)} = \frac{\text{No. of seeds germinated at 48 h}}{\text{No. of seeds germinated at 120 h}} \times 100$$

Co-efficient of germination and vigor index were calculated using the following formulae (Copeland 1976).

$$\text{Co-efficient of germination} = \frac{100 (A_1 + A_2 + \dots + A_n)}{A_1 T_1 + A_2 T_2 + \dots + A_n T_n}$$

$$\text{Vigor index} = \frac{A_1}{T_1} + \frac{A_2}{T_2} + \dots + \frac{A_n}{T_n}$$

Where,

A = Number of seeds germinated

T = Time (days) corresponding to A

n = No of days to final count

Shoot and root length, dry matter partitioning

At 5th day after placement for germination, five seedlings from each petridish were sampled, shoot and root length of individual seedling were recorded manually with scale. Then shoot, root and remaining seeds were dried separately at 70°C for 72 h and weight were recorded. The mean length (cm) and dry weight were calculated for each treatment combination.

Seed metabolic efficiency

Seed metabolic 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 respired. Thus higher the value of seed metabolic efficiency (SME), the higher is the efficiency of seed as more seed reserves would be used for producing roots and shoots.

Amount of seed material respired (SMR) was calculated as -

$$\text{SMR} = \text{SDW} - (\text{SHW} + \text{RTW} + \text{RSW})$$

Where,

SDW = Seed dry weight before germination

SHW = Shoot dry weight

RTW = Root dry weight

RSW = Remaining seed dry weight

Seed metabolic efficiency (SME) was calculated as-

$$\text{SME} = \frac{\text{SHW} + \text{RTW}}{\text{SMR}}$$

Statistical analysis

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

Experiment – II : Influence of post anthesis heat stress on physiology of grain in heat tolerant and heat sensitive wheat genotypes

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, 2001 to May, 2002 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 (Brammer, 1971 and Shaheed, 1984). As per USDA soil classification, the experimental soil was under Ochrept sub-order of Inceptisol order. The pH, CEC and C to N ratio of the soil were 6.5, 25.58 and 10:3, respectively. The information of basic physiochemical properties of the soil are presented in Appendix 1 and 2.

The experimental site is situated in the sub-tropical region characterized by heavy rainfall during the months from May to September and scanty rainfall in the rest of the year. Meteorological data on rainfall, temperature, relative humidity from December, 2001 to May, 2002 obtained from meteorological station of BSMRAU are presented in Appendix 3.

Experimental design and treatment

The experiment was conducted in a split plot design with four replications. The unit plot size was 3m x 2m 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 at November 30) and post anthesis heat stress condition (sowing at December 30) were placed in the main plots as main plot treatments whereas four wheat genotypes (three genotypes such as Aghrani, Kanchan and CB-30 having lower seed metabolic efficiency (SME) at 25°C and lower degree of reduction in SME both at 15 and 35°C and the other genotype Sonora having higher SME at 25°C and

greater reduction in SME both at 15 and 35°C) were placed randomly in the sub-plots as sub-plot treatments.

The treatment factors A and B were-

A. Main plot treatment: Two growing conditions

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

B. Sub-plot treatment : Four wheat genotypes

1. Aghrani
2. Kanchan
3. CB-30
4. 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 100-38-25-28 kg/ha N, P, K and S was applied in the form of Urea, Triple Super phosphate (TSP), Murate of potash (MP) and Gypsum, respectively. After land preparation, full dose of P, K and S 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 split into two equal amount Irrigation were given to avoid the drought stress when necessary.

Sowing

Seeds of four wheat genotypes (Aghrani, Kanchan, CB-30 and Sonora) were sown on November 30 and December 30, 2001 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 Thermostability (MT) test

Membrane thermostability was measured as described by Hossain *et al.* (1995). Flag leaf samples were collected at anthesis from five randomly selected plants of each genotypes and replication. 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). The leaf discs were washed three times with deionized water to remove electrolytes adhering to leaf tissue, as well as, electrolytes released from cut cells on the periphery of leaf discs. The test tubes (25 mm x 150 mm) were also rinsed with deionized water. Then ten leaf discs from five flag leaves were placed in a test tube and a piece of cotton was put on the

leaf discs inside the test tube to prevent any injury of the discs by the electrode bar during conductance measurement. There after 20 ml of deionized water was added in each tube.

The initial conductivity reading (I) was taken with an electric conductivity (EC) meter (Model SC 82, YOKOGAWA Electric Corporation, Japan). The test tubes were covered by aluminium foil and placed in a thermostatically controlled water bath incubator (Model BT-23, HITEC, Japan) maintaining a constant temperature of 55⁰C. Electrical conductivity reading (E) was also taken at 30 minutes interval up to 4.5 h, subsequently the samples were autoclaved at 121⁰C for 15 minutes to kill the leaf tissues completely. After autoclaving, the samples were again placed in water bath to adjust the elevated temperature of 55⁰C. Final conductance (F) was measured after 30 minutes of incubation. The percentage of injury induced by the elevated temperature during the time course (30 minutes) was calculated as follows.

$$\text{Injury (\%)} = \frac{E-I}{F-I} \times 100$$

Where,

I = The initial conductance

E = The elevated temperature conductance

F = The final conductance after autoclaving.

The time required to cause 50% injury at 55⁰C was defined as the heat killing time. Longer heat killing time in MT test indicated heat tolerance and shorter heat killing time indicated heat sensitivity of wheat genotypes.

Kernel growth rate and dry matter accumulation

At anthesis 70 ears were tagged from each plot. Five tagged ears were harvested to quantify grain growth at every 4th day beginning from anthesis. The harvesting of ears in all genotypes was continued up to 40 days after anthesis (DAA) for normal growing condition (November 30 sowing) and 36 DAA for post anthesis heat stress conditions (December 30 sowing). The harvested ears were kept in oven at 70⁰C for 72 hours. 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 an analytical balance (AND Electronic Balance Model ER 180A A & D Company Limited, Tokyo, Japan). The samples were shored 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.

Estimation of proline

Proline content of flag leaf and kernel at 8 days after anthesis in all wheat genotypes grown in two different growing conditions was estimated. At 8 days after anthesis, 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 one-gram fresh weight of both flag leaf and kernels were taken for proline estimation. Subsequently proline was estimated as Troll and Lindsley (1955).

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 30 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 ppm with distilled water.

The fresh samples were crushed in mortar and paste 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 and washed with 3% sulphosalicylic acid and the volume was set to 25 ml. 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 dispenser. 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. Proline content was expressed on a fresh weight basis from the standard curve, using standard L-proline according to the method developed by Trol and Lindsley (1955).

Estimation of soluble sugar and starch

The soluble sugar and starch content of grain at 4 days interval from anthesis in case of all wheat genotypes in both growing conditions were determined as Yoshida *et al.*, 1976.

Soluble sugar estimation: The dried composite grain 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 80-85⁰C for 30 minutes. Then the samples were centrifuged and decanted into a 50-ml beaker. This extraction was repeated three more times. The alcohol extract was evaporated on a water bath at 80-85⁰C 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, 1, 2, 3, 4, and 5 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/kernel 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°C 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 minutes 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/kernel 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.

Flag leaf collar height

Flag leaf collar height was measured from the base of the plant to the collar of the flag leaf. Ten plants sample from each treatment were taken and means were calculated.

Effective tillers per Plant

The number of effective tillers per plant was recorded at harvest. In that case ten plant sample from each treatment were taken randomly and means were calculated.

Heading, anthesis and maturity

Number of days from sowing to emergence of 50% heads and anthesis of 50% of the ears in each plot were estimated manually. Number of days from sowing to maturity was determined visually by the total loss of green color from the plant.

Spike character

Five spikes from the main shoot were collected randomly per plot to collect spike characters data.

1. Extrusion and spike length (excluding awn) were measured manually using scale.

2. Number of spikelets per spike, Number of florets per spike and number of kernels per spike were counted manually and floret sterility (%) was calculated by using the following formula-

$$\text{Floret sterility (\%)} = \frac{\text{No. of kernels /spike}}{\text{No of florets/spike}} \times 100$$

3. Then the collected spikes were dried in an oven at 70°C for 72 hours. After drying the spikes were weighed and grain were separated from husk and weighed.

Number of spikes, grain and straw dry wt/m²

The samples were collected from an area of 1m 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/m² 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-

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

Heat susceptibility index

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

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

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

($S < 0.5$, Highly stress tolerant; $0.5 < S < 1.0$, moderately stress tolerant and $S > 1.0$, stress susceptible)

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 Duncan's Multiple Range Test (DMRT) at 5% level of significance. In membrane thermostability test and kernel growth data only mean values were used and SE values were calculated.

CHAPTER IV

RESULTS AND DISCUSSION

The results of the two experiments- one conducted under laboratory condition and another under field condition have been presented in several Tables and Figures. Adequate discussion and possible interpretations whenever suitable have been provided.

Experiment-I : Influence of temperature regimes on seed reserve mobilization in wheat genotypes

4.1.1. Germination characteristics

Rate of germination was significantly influenced by the interaction effect of temperature regimes and wheat genotypes (Table 1). Co-efficient of germination and germination vigor index of the genotypes were influenced significantly by temperature regimes without expressing any interaction (Table 1). At 15⁰C temperature, the rate of germination was nil in all wheat genotypes i.e., none of the seeds were found to be germinated until 48 hr after placement for germination. At 25⁰C, all the wheat genotypes showed medium rate of germination (47.85 to 60.80%). The highest germination rate was found in CB-30 (98.89%) genotype at 35⁰C, which was statistically similar to all other genotypes except lower germination rate in Aghrani (75.14%).

Co-efficient of germination was increased with increasing temperature. The lowest value (24.53%) was found at 15⁰C in CB-24 genotype at 5th day, which was similar to all other wheat genotypes at 15⁰C. The highest value (28.54%) was found in

Table 1. Rate of germination, co-efficient of germination and germination vigor index in wheat genotypes as influenced by temperature regimes

Genotypes	Temp. (°C)	Rate of germination (%)	Co-efficient of germination (%)	Germination vigor index
Aghrani	15	-	24.61c	20.77 c
	25	47.85 e	27.20b	29.63 b
	35	75.14 c	28.15a	33.87 a
Kanchan	15	-	24.52 c	14.80 c
	25	53.87 de	26.81 b	28.83 b
	35	90.96 ab	28.28 a	34.76 a
CB-30	15	-	24.82 c	22.41 c
	25	53.71 de	26.61 b	29.43 b
	35	98.89 a	28.54 a	37.48 a
Sonora	15	-	24.91c	21.84 c
	25	60.80 d	27.08 b	30.90 b
	35	98.85 a	28.54 a	36.36 a
CB-24	15	-	24.53c	18.96 c
	25	58.97d	26.99b	29.75b
	35	88.66 b	28.30a	33.33 a
CB-34	15	-	24.55 c	20.33 c
	25	54.68 de	26.90b	30.12 b
	35	93.06 ab	28.26 a	35.83 a
CV(%)		10.84	1.65	7.2

Means followed by the same letter (s) did not differ significantly at %5 level by DMRT

Sonora, which was also statistically similar to all other genotypes at 35⁰C. At 25⁰C, all the genotypes showed medium value (26.61 to 27.20%).

Germination vigor indices showed an increasing trend with increasing temperature. The lower indices (14.80 to 30.90) were found at 15⁰C, which increased to moderate level (28.83 to 30.90) at 25⁰C and then to the highest level (33.33 to 37.48) at 35⁰C in all wheat genotypes. However, the germination vigor indices increased with greater extend as the temperature rises from 15⁰C to 25⁰C, which with further increase in temperature up to 35⁰C increased with lesser extent.

Faster speed of germination at higher temperature might be due to rapid hydrolysis and mobilization of seed reserves through higher alpha amylase activity at higher temperature. The close relation between germination of wheat seed and alpha amylase activity at various temperatures was reported by Sultana *et al.* (2000). In respect to speed of germination all wheat genotypes behaved almost similar at different temperature and genotypic differences in speed of germination due to increase in temperature did not appear clearly.

4.1.2. Height and dry matter of seedling

The length and dry weight of shoot and root of wheat seedling were influenced significantly by the interaction effect of temperature and wheat genotypes (Table 2). The shoot length was the lowest in Kanchan (1.14 cm) which was statistically comparable to all other genotypes at 15⁰C. Shoot length increased (5.69 to 9.03 cm) at 25⁰C which was considered to be optimum temperature for wheat seedling growth. At 35⁰C temperature,

shoot length remained more or less same (7.53 cm) in Aghrani as in 25⁰C and increased in Kanchan (6.69 cm) and CB-30 (8.01 cm). But in the remaining wheat genotypes (Sonora, CB-24 and CB-34), it was decreased (6.23 to 8.78cm) as compared to that of 25⁰C.

In case of root length, the lowest value was attained at 15⁰C, it increased at 25⁰C and thereafter decreased at 35⁰C in all wheat genotypes. At 35⁰C, the reduction in root length from that at 25⁰C was lower (2.84 to 4.74 cm) in Aghrani, Kanchan and CB-30 than that of Sonora, CB-24 and CB-30 (5.93 to 6.37 cm). The adverse effect of higher temperature (35⁰C) on root length was more clearly noted than that on shoot length.

The shoot to root ratio of length increased with increasing temperature regimes in all wheat genotypes. It was low at 15⁰C (0.33 to 0.42), moderate at 25⁰C (0.59 to 1.05) and high at 35⁰C (1.26 to 1.84). The increasing shoot to root ratio of length with increasing temperature regimes indicated that root was more affected than shoot at high temperature.

Generally, shoot dry weight increased significantly with increasing temperature, in all wheat genotypes, except in CB-34 at 35⁰C. The increment of shoot dry weight from 25⁰C to 35⁰C was higher in Aghrani, Kanchan and CB-30 (1.27 to 1.48 mg/seedling) than that of Sonora (0.31 mg/seedling) and CB-24 (0.77 mg/seedling), whereas in CB-34, it was decreased. In case of root dry weight, higher value was found at 25⁰C and it was reduced significantly both at 15 and 35⁰C in all wheat genotypes.

The shoot to root dry weight ratio increased with increasing temperature in all wheat genotypes. Increment of ratio of shoot to root dry weight with increasing

Table 2. Length and dry weight of shoot and root of wheat seedlings as influenced by temperature regimes

Genotypes	Temp. (⁰ C)	Length (cm)			Dry weight (mg)		
		Shoot	Root	Shoot: Root	Shoot	Root	Shoot: Root
Aghrani	15	1.28 f	3.07g	0.41 h	1.19g	2.03 gh	0.60 e
	25	7.80 abc	7.41 c	1.05 d	5.87ef	4.13 cde	1.34 cd
	35	7.53 bcd	4.57 ef	1.68 b	7.01 cde	2.91 fgh	2.42 a
Kanchan	15	1.14f	3.10 g	0.37h	1.00g	2.08 gh	0.44 e
	25	5.69 e	9.93 b	0.59 g	6.53 def	5.51 b	1.19 d
	35	6.69 cde	5.35 e	1.35c	8.01abc	3.99 cde	2.01 b
CB-30	15	1.25 f	3.00 g	0.42 h	1.05 g	2.19 gh	0.49 e
	25	7.22 cd	11.15 a	0.64 g	6.20 ef	4.82 bc	1.30 cd
	35	8.01 fg	6.41 d	1.26 c	7.47bcd	3.62 def	2.03 b
Sonora	15	1.31 f	3.12 g	0.42 h	1.09g	1.89 h	0.60 e
	25	9.03 a	11.57 a	0.85 e	8.35 ab	6.76a	1.24 d
	35	8.78 ab	5.60 de	1.57 b	8.66a	4.61 bcd	1.84 b
CB-24	15	1.07 f	2.80 g	0.38 h	1.07 g	1.85 h	0.55 e
	25	7.23 cd	9.42 b	0.77 ef	5.80f	4.28 cde	1.33 cd
	35	6.23 de	3.37 g	1.84 a	6.57def	3.39 ef	1.94 b
CB-34	15	1.21 f	3.71 fg	0.33 h	1.07 g	1.87 h	0.56 e
	25	7.72 abc	11.63 a	0.66fg	6.73def	5.57 b	1.13 d
	35	7.09 cd	5.26 e	1.35 c	5.79 f	3.18 efg	1.50 c
CV(%)		13.49	9.73	8.28	11.92	12.12	11.41

Means followed by the same letter (s) did not differ significantly at %5 level by DMRT

temperature indicated that root dry weight was reduced but shoot dry weight increased at higher temperature. Based on increment of shoot to root dry weight ratio from 25⁰C to 35⁰C Aghrani, Kanchan and CB-30 showed higher increment in shoot dry weight (0.73 to 1.08mg) than Sonora, CB-24 and CB-34 (0.37 to 0.61mg).

4.1.3. Seed dry matter distribution

The percent seed dry matter accumulated in shoot and root, the amount of dry matter loss during respiration and the dry matter remained in seed at 5 days after placement for germination at 15, 25 and 35⁰C showed clear differences among the genotypes (Table 3 and Figure 1). The amount of seed reserve lost due to respiration increased from 25⁰C to 35⁰C in all wheat genotypes, but the increment was higher in Sonora (14.1%) and CB-24 (13.19%), moderate in Kanchan (6.36%) and CB-34 (4.95) and lowest in Aghrani (2.03%). At 15⁰C temperature, the amount of respiratory loss of seed reserve was also increased than that at 25⁰C in all genotypes, except in Aghrani and CB-30. But again the increment was higher in Sonora, CB-24 and CB-34 (2.79 to 8.84%) than that of Kanchan (1.67%). In case of Aghani and CB-30, it was even decreased.

At low temperature (15⁰C), the proportion of dry matter distributed to shoot (2.37 to 3.28%) and root (4.7 to 5.81%) was lower in all genotypes compared to those at any higher temperature (25⁰C to 35⁰C). Dry matter accumulation in shoot weight was increased significantly over a larger increase in temperature (15⁰C to 35⁰C). But dry matter accumulation in root was increased over a smaller range of increasing temperature (15⁰C to 25⁰C) and thereafter decreased at 35⁰C in all wheat genotypes. The results indicated that the optimum temperature for root growth was lower compared to that for

Table 3. Seed dry matter distribution (%) at 5 days after placement for germination in different wheat genotypes as influenced by temperature

Genotypes	Temp. (°C)	Distribution of seed dry matter (%)			
		In shoot	In root	Respired	Remain in seed
Aghrani	15	3.05 f	5.25 gh	9.18jk	82.52 a
	25	13.85 e	10.34 cd	13.84 fgh	61.95 cd
	35	16.89 bcd	6.87 fg	15.87 dfg	60.70 de
Kanchan	15	2.37 f	4.70h	15.71defg	77.36 ab
	25	15.06 de	12.76 b	14.04efgh	58.15 def
	35	18.31 ab	9.12 de	20.40 b	52.17g
CB-30	15	2.74 f	5.72 gh	13.31ghi	78.18 ab
	25	16.04 cd	12.50b	14.37 efgh	57.08 defg
	35	17.39 bc	8.71 de	19.52 bc	53.71 fg
Sonora	15	2.64 f	4.30h	18.05 cd	75.02 b
	25	19.71 a	15.52 a	11.19 ij	53.92 fg
	35	18.80 ab	10.00 cd	25.29 a	45.92 h
CB-24	15	3.07 f	5.27gh	16.42 de	75.25b
	25	15.11 de	11.14 bc	7.58 k	66.17 c
	35	15.03 de	7.74 ef	20.77 b	56.46 efg
CB-34	15	3.28 f	5.81 gh	15.69 defg	75.21b
	25	17.74 bc	15.62 a	12.90 hi	53.74 fg
	35	18.19 ab	9.82 cd	17.95 cd	54.45 fg
CV(%)		8.78	11.65	8.36	4.72

Means followed by the same letter (s) do not differ significantly at %5 level by DMRT

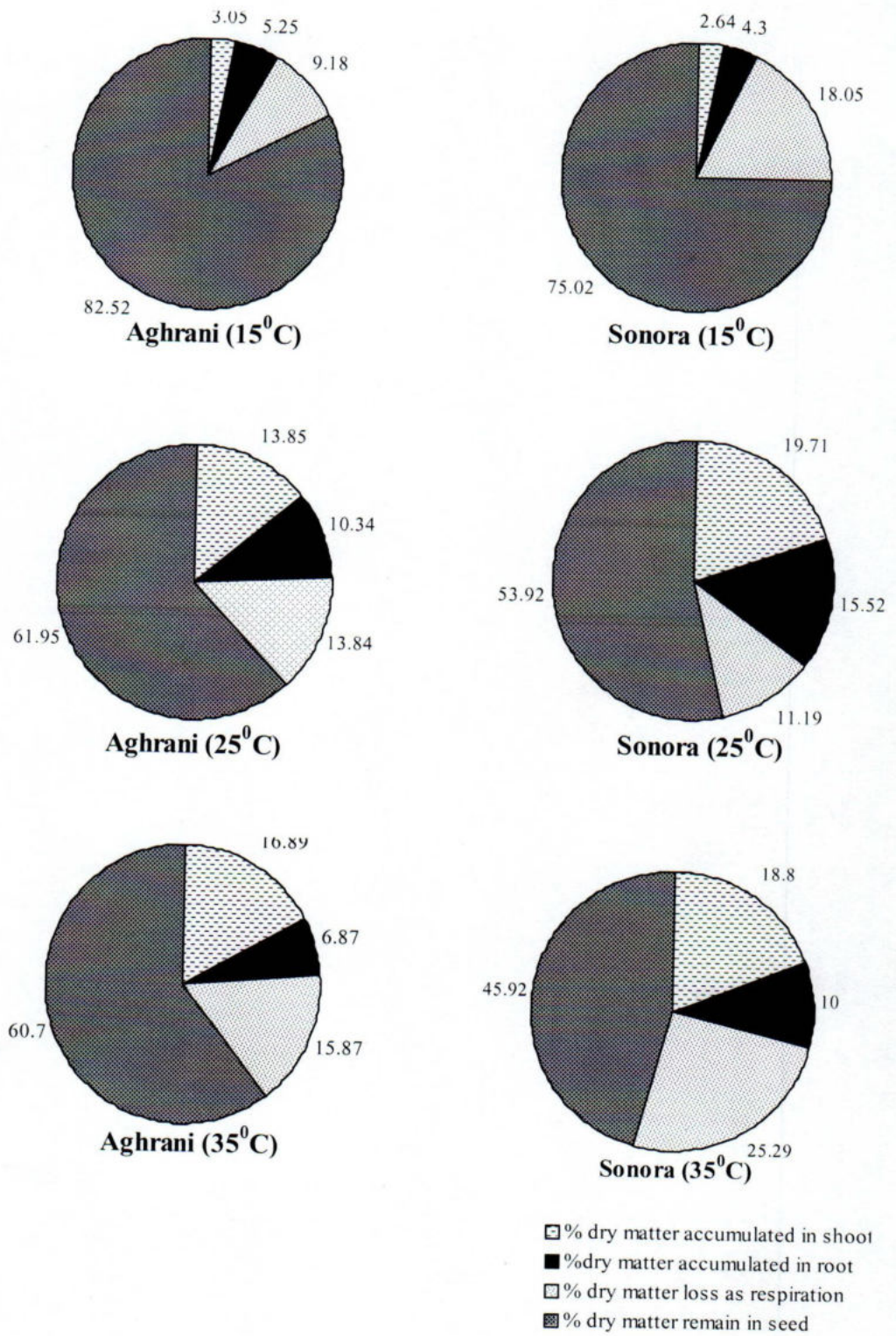


Figure 1. Percent dry matter distribution at 5 days after placement for germination in Aghrani and Sonora

shoot growth. Therefore, when root failed to continue to increase dry matter at high temperature (35⁰C), the shoot was able to continue to gain dry matter showing it higher temperature optima than root.

Finally distribution of seed reserves into different parts of 5-day old seedling have been shown using the variety Aghrani having less affected root and shoot characters under high temperature (35⁰C) and another variety Sonora representing more adversely affected root and shoot characters (Figure 1). At 25⁰C temperature, both Aghrani and Sonora translocated its seed reserves into seedling more efficiently with minimum respiratory loss of seed reserves (Figure 1). However, both at 15⁰C and 35⁰C temperature, Sonora did not accumulate its seed reserves into seedling as efficiently as Aghrani.

4.1.4. Seed metabolic efficiency (SME)

Temperature had a profound effect on seed metabolic efficiency (SME) in all wheat genotypes (Figure 2). At 25⁰C temperature, the SME was significantly higher than that at 15⁰C and 35⁰C in all wheat genotypes. Sonora, CB-24 and CB-34 showed higher SME (2.58 to 3.48 g/g) than other genotypes (1.99 to 2.09 g/g) at 25⁰C. Based on the amount of reduction in SME both at 15⁰C and 35⁰C compared to that at 25⁰C, the genotypes Sonora, CB-24 and CB-34 were experienced larger reduction (1.04 to 2.69g/g) than Aghrani, Kanchan and CB-30 (0.60 to 1.70g/g).

The lowest SME at 15⁰C temperature suggested that at low temperature respiration was not linked to build up shoot and root biomass and could have led to thermal dissipation of the respiratory energy by the way of alternate oxidase pathway or cyanides resistant pathway as suggested by Henry and Nyns (1975) and Day *et al.* (1980).

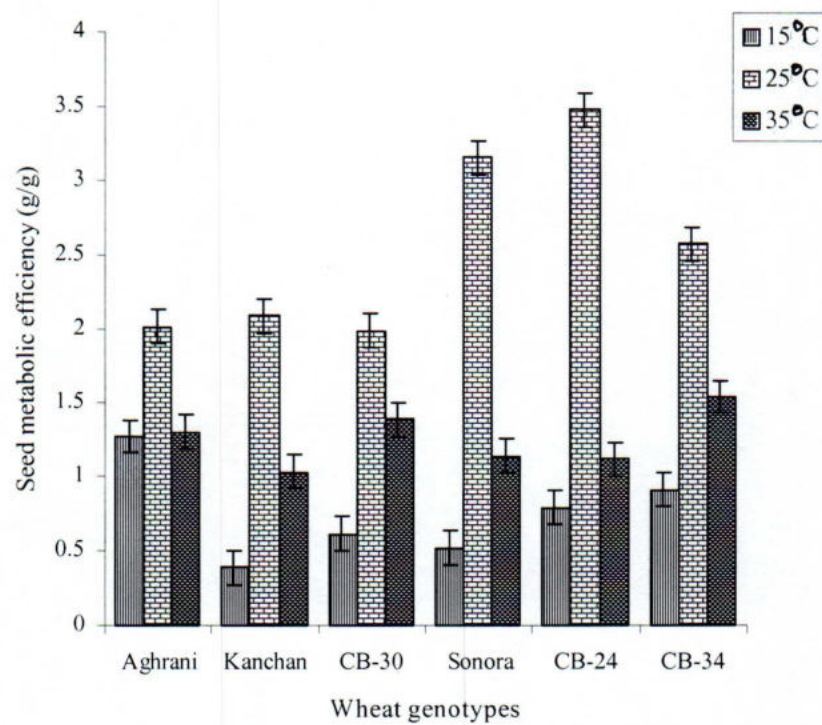


Figure 2. Seed metabolic efficiency in wheat genotypes as influenced by temperature

Again at high temperature (35°C) reduction in SME under high temperature could be due to increased rate of respiration but the respiratory product somehow failed to accumulate in seedling, mainly in root. Here, based on degree of reduction in SME either at lower temperature (15°C) or at higher temperature (35°C) compared to the normal temperature (25°C) genotypes of wheat can be arranged in different groups. Three genotypes (Aghrani, Kanchan and CB-30) having lower SME at 25°C and lower reduction in SME at 15 and 35°C , on the other hand Sonora having higher SME at 25°C and greater reduction both at 15 and 35°C were used field experiment.

Experiment – II: Influence of post anthesis heat stress on physiology of grain in heat tolerant and heat sensitive wheat genotypes

The field experiment was conducted to study the influence of post anthesis heat stress on physiology of grain in heat tolerant and heat sensitive wheat genotypes. All the wheat genotypes sown in the field at November 30 exposed to $<26^{\circ}\text{C}$ during the reproductive growth phase (Figure 3 and 4) which was very close to normal temperature of $22\text{--}26^{\circ}\text{C}$ required for reproductive growth phase (Al-Khatib and Paulsen, 1990 and Campbell and Read, 1968) and considered as normal growing condition. On the other hand when they were sown at December 30 experienced high temperature ($>26^{\circ}\text{C}$) during reproductive phase (Figure 3 and 4) and regarded as post anthesis heat stress condition. Under normal growing condition, Aghrani, Kanchan, CB-30 and Sonora exposed to average mean air temperature of 23.78, 24.36, 24.66 and 22.60°C , respectively during anthesis to maturity. On the other hand, at post anthesis heat stress condition, Aghrani, Kanchan and CB-30 exposed to 2.44, 2.02 and 1.72°C more average mean air temperature, respectively, whereas Sonora received 3.05°C more average mean air temperature from normal growing condition during anthesis to maturity (Figure 5).

4.2.1. Cell membrane thermostability (CMT)

Cell membrane thermostability (CMT) as determined by relative electrolyte leakage at 55°C elevated temperature differed significantly among the wheat genotypes under both normal and post anthesis heat stress conditions (Table 4). Time required to reach 50% membrane leakage in a genotype was considered to be its heat killing time. The longest heat killing time (150 min.) was observed in Aghrani and it was closely

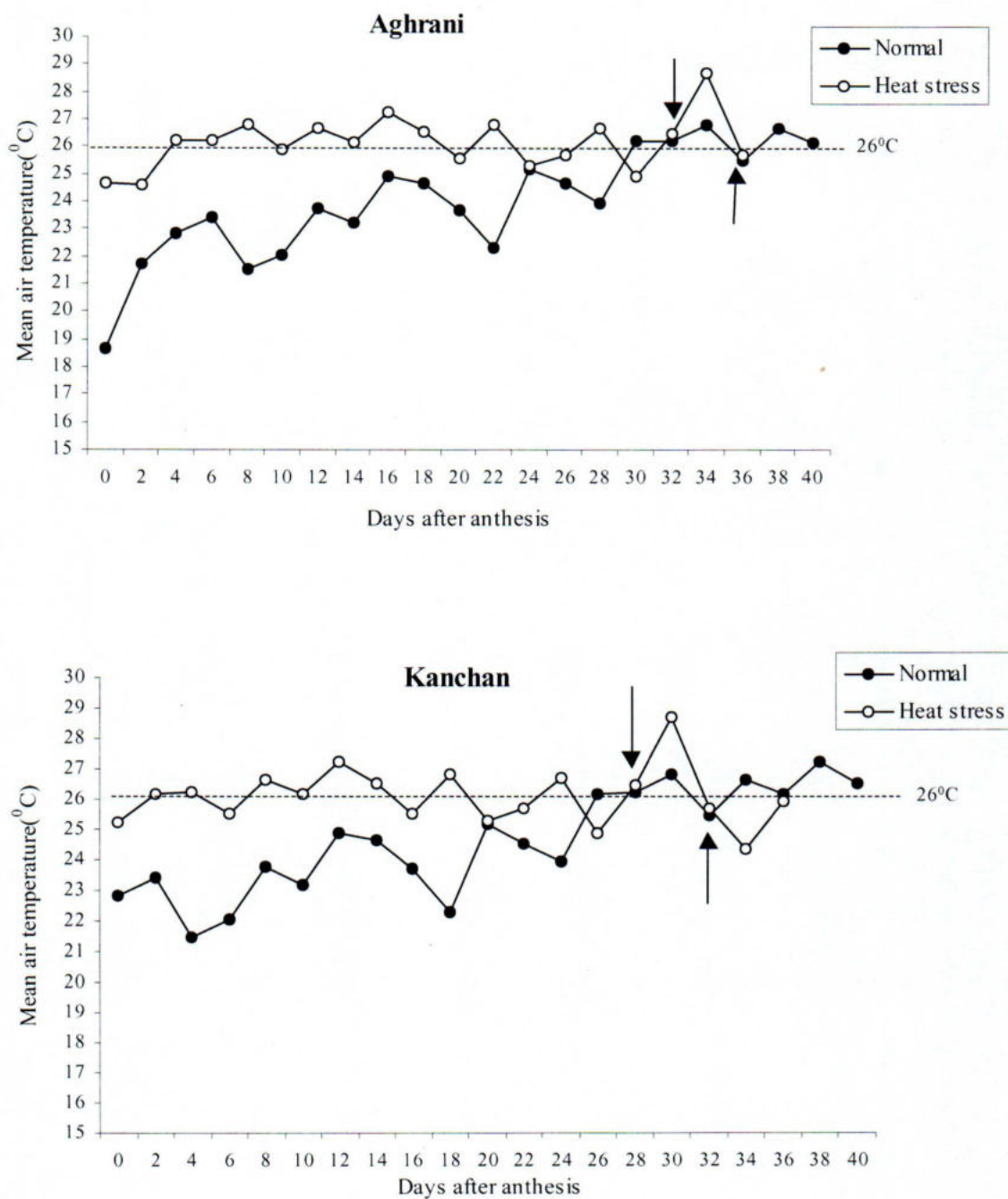


Figure 3. Mean air temperature received by Aghrani and Kanchan from anthesis to maturity under normal and post anthesis heat stress conditions (→ indicates physiological maturity).

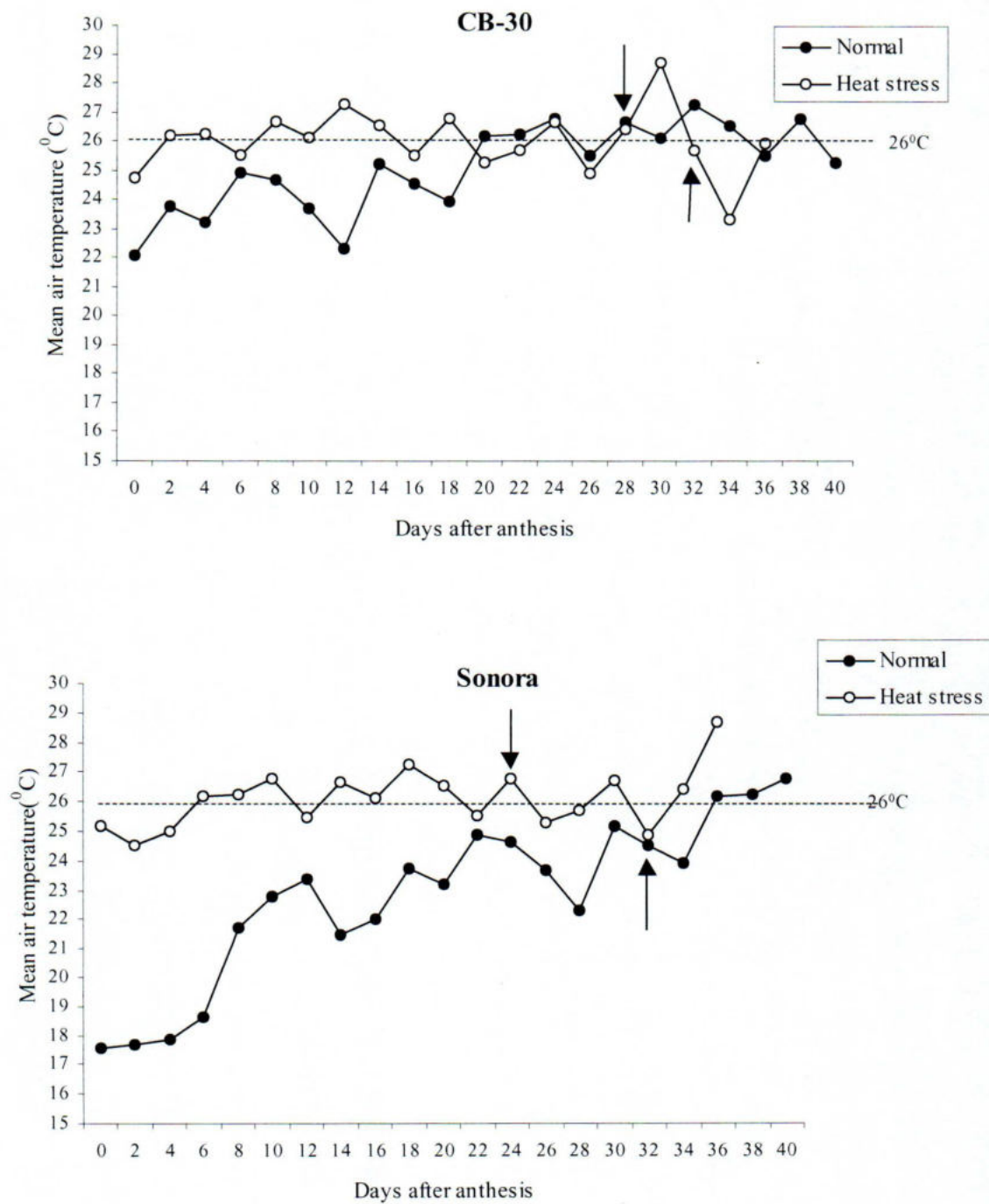


Figure 4. Mean air temperature received by CB-30 and Sonora from anthesis to maturity under normal and post anthesis heat stress conditions (—► indicates physiological maturity).

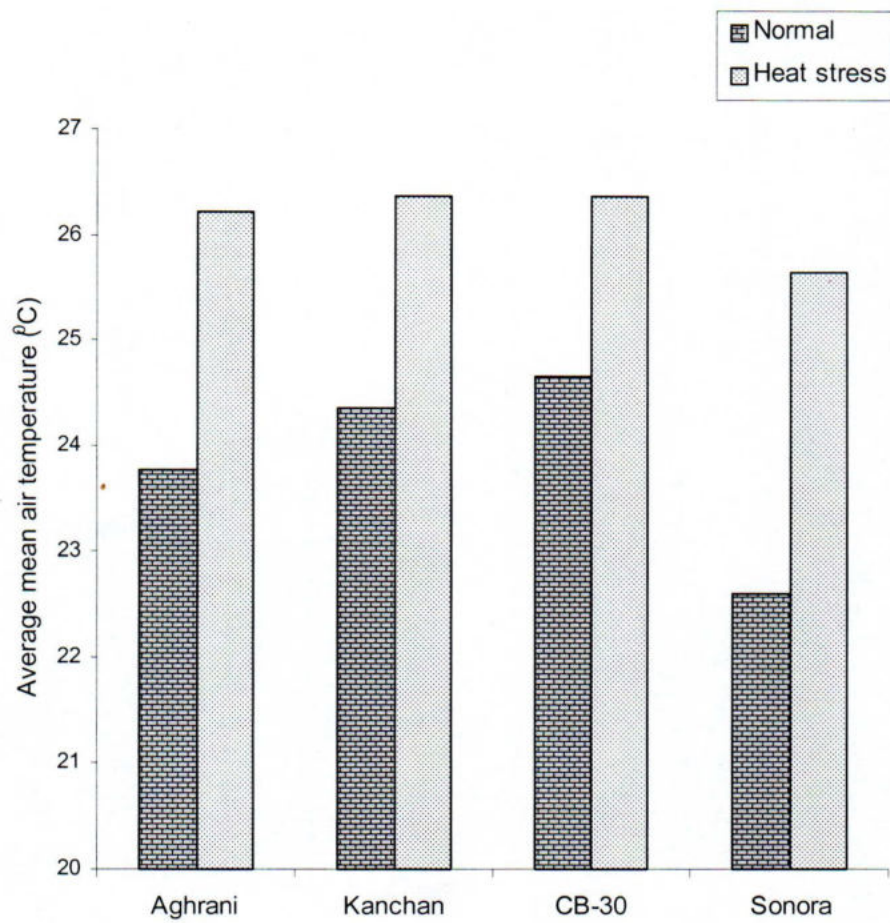


Figure 5. Average mean air temperature ($^{\circ}\text{C}$) received by different wheat genotypes during anthesis to maturity under normal and post anthesis heat stress conditions

Table 4. Relative heat tolerance of wheat genotypes as determined by percent membrane leakage (mean $\pm$ SE) of flag leaf tissue and time to attain that injury

Genotypes	Growing conditions	Membrane leakage (%) at 55°C temperature								
		30 min.	60 min.	90 min.	120 min.	150 min.	180 min.	210 min.	240 min.	270 min.
Aghrani	Normal	28.79 ± 0.85	38.73 ± 0.65	43.12 ± 0.53	48.50 ± 0.53	50.85 ± 0.33	53.01 ± 0.16	56.87 ± 1.09	68.54 ± 1.14	74.83 ± 0.63
	Heat stress	28.07 ± 0.75	36.69 ± 0.22	42.33 ± 0.62	48.26 ± 0.39	50.22 ± 0.20	51.54 ± 0.47	55.65 ± 1.00	67.97 ± 0.76	73.88 ± 0.82
Kanchan	Normal	37.37 ± 0.42	41.45 ± 0.46	48.35 ± 0.42	51.24 ± 0.25	56.68 ± 0.43	62.37 ± 0.59	72.82 ± 0.18	77.21 ± 0.93	81.23 ± 0.63
	Heat stress	35.55 ± 0.58	40.18 ± 0.33	46.04 ± 0.65	50.29 ± 0.27	56.96 ± 0.65	60.87 ± 1.08	71.57 ± 0.55	76.41 ± 0.93	80.44 ± 1.02
CB-30	Normal	36.15 ± 0.57	44.57 ± 0.39	48.07 ± 0.49	50.41 ± 0.42	56.04 ± 0.83	64.23 ± 0.99	72.64 ± 0.73	78.15 ± 1.03	83.14 ± 0.58
	Heat stress	33.15 ± 0.53	43.15 ± 0.69	46.82 ± 0.42	49.89 ± 0.22	55.54 ± 0.85	63.73 ± 0.76	71.39 ± 0.52	77.40 ± 0.57	81.64 ± 0.71
Sonora	Normal	51.49 ± 1.14	57.03 ± 0.75	62.10 ± 0.85	69.73 ± 0.84	75.22 ± 0.25	78.13 ± 0.69	80.86 ± 0.60	84.71 ± 0.54	86.92 ± 0.32
	Heat stress	51.50 ± 0.76	57.53 ± 0.44	62.26 ± 0.66	70.05 ± 0.75	75.82 ± 0.60	78.63 ± 0.60	81.42 ± 0.70	85.13 ± 0.42	86.41 ± 0.25

followed by Kanchan (120 min.) and CB-30 (120 min.) both under normal and post anthesis heat stress conditions. Among the genotypes Sonora showed the shortest heat killing time (30 min.) irrespective of growing conditions. Therefore, under post anthesis heat stress condition, the genotypes Aghrani, Kanchan and CB-30 showed somewhat lower membrane leakage at any particular time after exposure to elevated temperature than that under normal condition. This could be because of the tendency of the genotypes to acclimatize against high temperature stress. In Sonora almost unchanged membrane leakage both under normal and heat stressed condition at a particular time indicated the absence of acclimation ability of this genotype.

Based on heat killing time, Aghrani, Kanchan and CB-30 were considered as heat tolerant (HT) genotypes while Sonora as heat sensitive (HS) genotype.

Under heat stress, the activity of superoxide di-mutase and catalase increased more in heat tolerant wheat genotypes than in sensitive one (Zhou *et al.*, 1995). As the high temperature membrane injury was caused by free radicals, the higher activity of superoxide di-mutase and catalase may be important in the higher membrane thermostability in tolerant cultivars. Genotypic differences in membrane thermostability was also reported in wheat by Fokar *et al.* (1988), Shanahan *et al.* (1990), Sadalla *et al.* (1990), Sharma and Tandon (1998), Sikder (1999), Blum *et al.* (2001) and Haque (2002) and in cabbage by Hossain *et al.* (1995).

4.2.2. Proline content

Proline content in flag leaves and kernels of different wheat genotypes at 8 days after anthesis was significantly influenced by post anthesis heat stress conditons (Table 5).

Table 5. Flag leaf and kernel proline content in wheat genotypes at 8 days after anthesis under normal condition and post anthesis heat stress condition

Genotypes	Flag leaf proline content ($\mu\text{mole/g}$ fresh wt.)			Kernel proline content ($\mu\text{mole/g}$ fresh wt.)		
	Normal condition	Heat stress condition	Relative change to normal (%)	Normal condition	Heat stress condition	Relative change to normal (%)
Aghrani	0.69 e	0.79 d	+14.49	1.59 d	1.25 e	-21.38
Kanchan	0.96 c	1.25 a	+30.20	1.95 c	1.20 e	-38.46
CB-30	0.87 d	1.28 a	+47.13	2.47 b	2.41 b	-2.43
Sonora	0.50 f	1.11 b	+122.00	3.58 a	1.89 c	-47.21
CV(%)	5.38		-	3.77		-

Means followed by the same letter (s) did not differ significantly at %5 level by DMRT

Proline content in the flag leaves was significantly higher in heat tolerant genotypes e.g. in Aghrani (0.69 $\mu\text{mole/ g. fresh wt.}$), Kanchan (0.96 $\mu\text{mole/ g. fresh wt.}$) and CB-30 (0.87 $\mu\text{mole/ g. fresh wt.}$) than that in heat sensitive Sonora (0.50 $\mu\text{mole/ g. fresh wt.}$) under normal growing condition. Relative changes due to heat stress indicated that there was significant increase in flag leaf proline of wheat compared to normal condition. But the per cent increment was lower (14.49% to 47.13%) in heat tolerant genotypes than in heat sensitive genotype (122%).

Again, under normal growing condition, the kernel proline content at 8 days after anthesis was recorded to be significantly lower (1.59 to 2.47 $\mu\text{mole/ g}$) in heat tolerant genotypes (Aghrani, Kanchan and CB-30) than in heat sensitive Sonora (3.58 $\mu\text{mole/ g}$). Although kernel proline content was lower under post anthesis heat stress condition in all genotypes but the reduction was not significant in CB-30. The per cent reduction of kernel proline content was lower (2.43% to 38.46%) in heat tolerant genotypes than in heat sensitive Sonora (47.21%). Relative to normal growing condition the sharp increase in flag leaf proline along with larger decrease in kernel proline under heat stress condition clearly indicate the sensitivity of the genotype (Sonora) to post anthesis heat stress. In other word, smaller changes in proline level due to heat stress was observed in heat tolerant genotypes. These results are in agreement with those of Maiti *et al.* (2000) in various crops, Chaitanya *et al.* (2001) in Mulberry leaves, Talwar *et al.*, (1999) in Groundnut, Takeda *et al.*, (1999) and Hossain *et al.*, (1995) in Brassica and Zhang *et al.*, (1998) in Cucumber.

Kernel dry matter accumulation

Kernel dry matter accumulation followed more or less similar pattern in three heat tolerant (HT) genotypes i.e. Aghrani, Kanchan and CB-30, the heat sensitive (HS) genotype Sonora both under normal and post anthesis heat stress conditions (Figure 6). Both under normal and post anthesis heat stress conditions, a typical sigmoidal pattern of dry matter accumulation was discernible in all genotypes. Under normal growing condition, the dry weight in Aghrani was observed to be increased up to 53.69 mg/kernel at 36 days after anthesis (DAA) and declined thereafter slowly. In other two HT genotypes i.e. in Kanchan and CB-30, the dry weight was found to be increased up to 51.35 & 49.62 mg/ kernel, respectively at 32 days after anthesis and declined thereafter. The HS genotype Sonora showed similar pattern and attained the highest dry weight (53.04 mg/seed) at 32 days after anthesis.

Under post anthesis heat stress condition, maximum dry matter accumulation per kernel and duration to attain that kernel dry matter were reduced in all wheat genotypes. The extent of reduction in dry matter accumulation in heat tolerant Aghrani (9.0%), Kanchan (8.5%) and CB-30 (6.59%) was closely followed by slightly higher reduction rate in the HS Sonora (10.25%). Again, the reduction in duration required to attain the maximum kernel was 4 days in the HT genotypes whereas it was reduced by 8 days in the Sonora.

The declining tendency in dry matter after attaining the highest level could be due to respiratory loss of kernel. Thus it indicates the importance of harvesting the crop and quickly drying the wheat grains when it attains physiological maturity. Sigmoidal pattern of seed dry matter accumulation was also found by Chanda *et al.* (1999) in wheat.

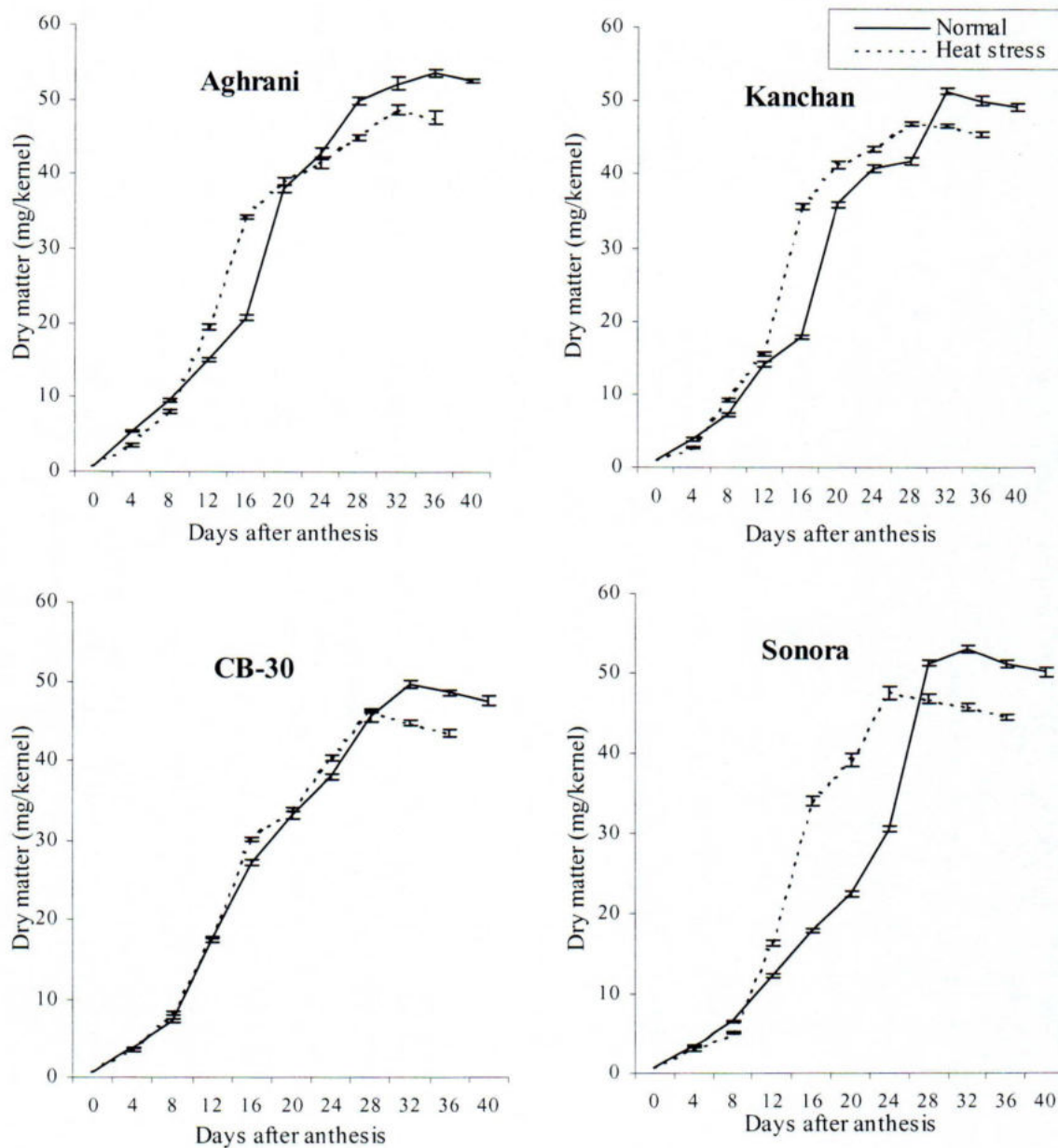


Figure 6. Kernel dry matter accumulation in different wheat genotypes at different days after anthesis under normal and post anthesis heat stress conditions

4.2.4. Kernel Growth rate

The absolute growth rate and duration of rapid growth rate (more than 1 mg/day/kernel) revealed significant differences among the genotypes and between the growing conditions (Figure 7). Under normal growing condition, all the genotypes maintained a longer duration of rapid growth rate, apparently 22-30 days. But under post anthesis heat stress condition, this duration was reduced. The HT genotypes possessed this duration of rapid growth rate of about 16-28 days whereas the HS genotype, Sonora, maintained that growth rate only for 10 days. These results agreed with those of Bhatta *et al.* (1994). They found that delayed sowing caused a high temperature stress condition at grain filling period resulting reduced grain filling period and rate but the reductions were lower in tolerant varieties than those of sensitive varieties. Wiegand and Cuellar (1981), Bruckner and Fruhberg (1987), Jhala and Jadon (1989), Sikder *et al.* (1999) and Sarker *et al.* (2001) made similar statement.

The grain 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. Then grain growth depend largely on it's the stored food materials mobilizing from other plant parts, thus enhancing the whole plant senescence. Under normal condition, all genotypes were delayed to initiate the phase of internal starvation and, as a result, had longer duration of rapid growth than heat stress. At post anthesis heat stress condition, they initiated starvation phase earlier and reduce the rapid growth duration. But the HS genotype, Sonora because of its earliest cessation of rapid growth rate had minimum grain growth duration.

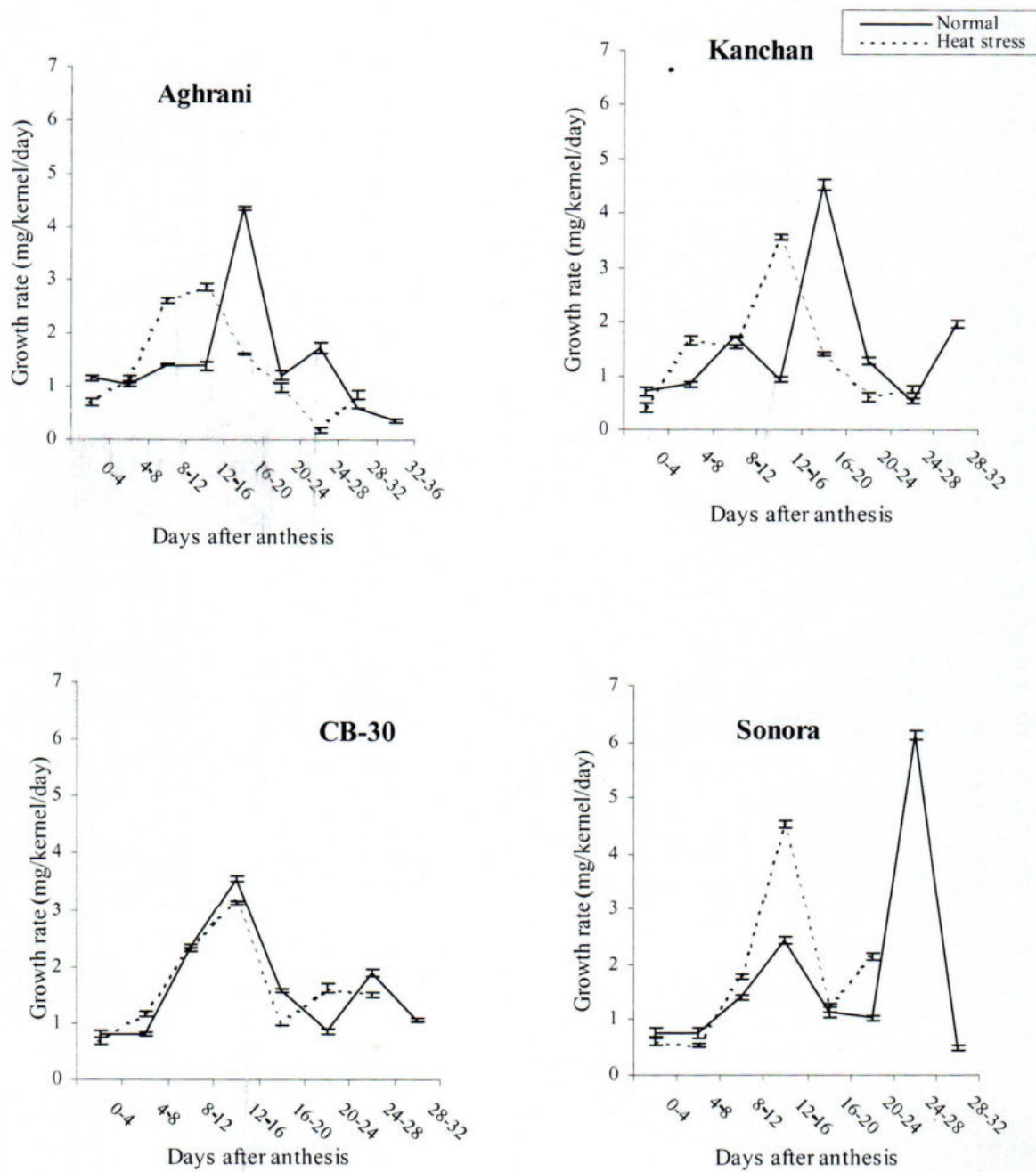


Figure 7. Kernel growth rate in different wheat genotypes at different days after anthesis under normal and post anthesis heat stress conditions

4.2.5. Starch accumulation pattern in kernel

Starch contributed up to 74% of the kernel dry matter and thus considered as the major contributor to the kernel dry matter accumulation during development (Appendix 5). In the present experiment starch accumulation followed a sigmoidal pattern in tested wheat genotypes and in both growing conditions (Figure 8). Under normal growing condition the starch accumulation pattern reached the highest level at 32 days after anthesis (DAA) in HT genotypes, Kanchan and CB-30 and in the HS genotype Sonora. Another HT genotype Aghrani reached the maximum starch accumulation stage at 36 DAA. This result revealed that no clear difference between HT and HS genotypes in respect to duration of maximum starch level under normal condition. But under heat stress condition data showed not only clear reduction in days to reach the peak level of starch accumulation but also indicate variations between HT and HS genotypes. Days to reach the peak starch accumulation were reduced by 4 days in HT genotypes (e.g., Aghrani, Kanchan, CB-30) whereas such reduction was extended to 8 days in HS genotypes. It is clear here that times to reach the maximum dry matter and times to reach the highest starch in kernel coincided and hence rate of starch in kernel dry matter accumulation became evident. Chanda *et al.* (1999) also found a close correspondence between these two parameters.

4.2.6. Soluble sugar content in kernel

Soluble sugar content in kernel at different days after anthesis varied between normal condition and heat stress conditions in all wheat genotypes (Figure 9). Under normal growing condition, total soluble sugar was higher during early stages, declined

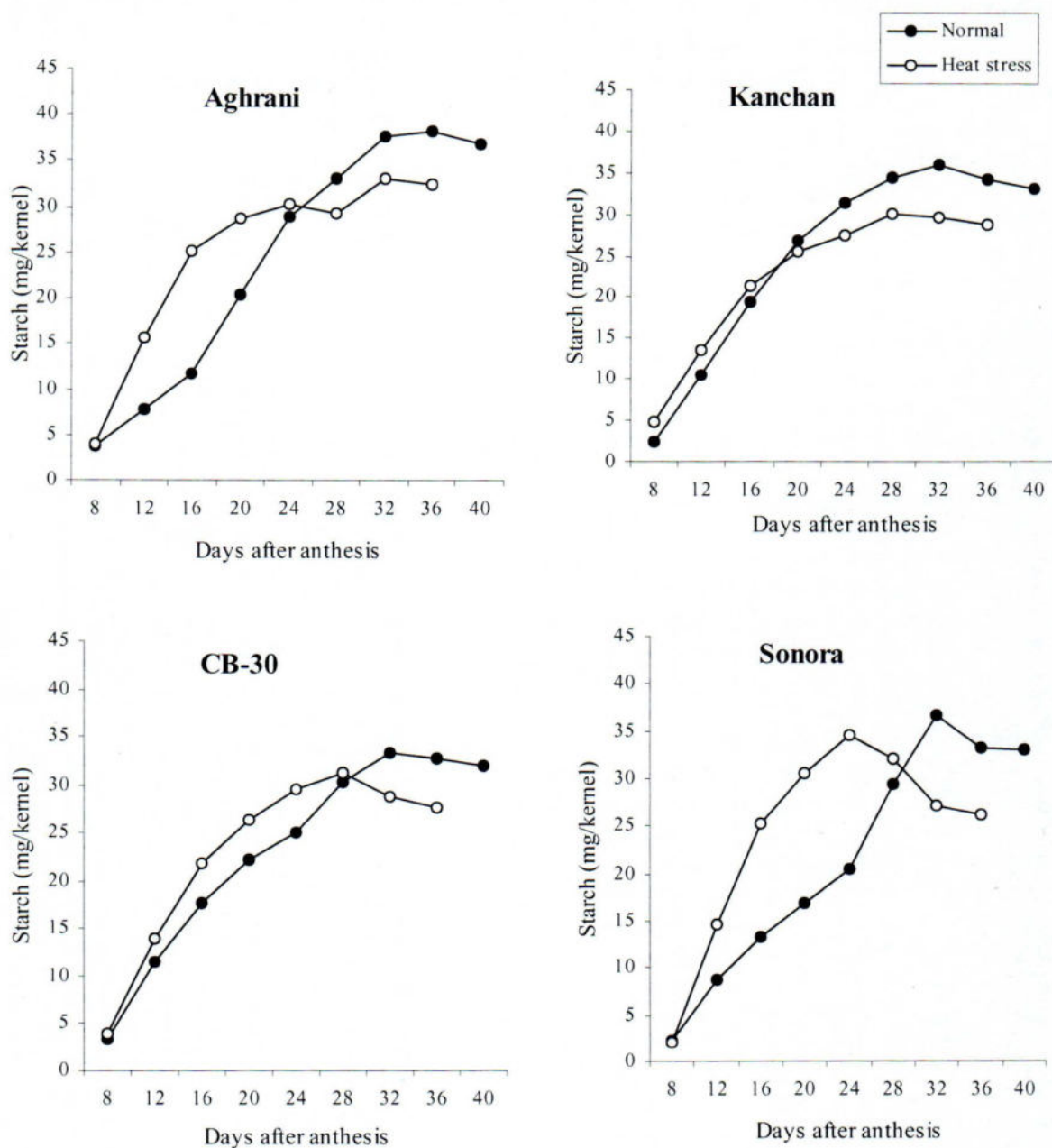


Figure 8. Starch content in wheat genotypes at different days after anthesis under normal and postanthesis heat stress conditions

with increasing demand for starch synthesis up to the middle part of the kernel growth and thereafter increased at later stages in both HT and HS genotypes. Under heat stress condition, though the sugar content was lower throughout the whole kernel developing period, the HT genotypes Aghrani, Kanchan and CB-30 showed similar trend as under normal condition. On the other hand, in HS genotype Sonora sugar content/kernel under heat stress condition was lower during early stages, increased with increasing growth up to 24 DAA and thereafter declined sharply with the start of maximum rate of dry matter accumulation. Similar trend of soluble sugar content in wheat was also reported by Chanda *et al.* (1999). The results revealed that the availability of soluble sugar might become the limiting factor during the rapid kernel-growing period in all genotypes under normal condition. In heat stress condition although the HT genotypes showed the similar phenomenon as under normal condition but the HS genotype showed a different pattern of soluble sugar content in kernel. In kernel of HS genotype the early declination of dry matter accumulation and rapid kernel growth rate altogether resulted in the rapid increase in soluble sugar under heat stress condition. This happened apparently due to limited conversion of sugar into starch rather than limited supply of sugar in kernel. One of the speculations might be the limited enzymatic function involving in sugar to starch conversion step, which needs further experimentation to confirm. However, many authors reported that regulation of starch synthesis in the cereal endosperm has been suggested to be at glucose pyrophosphorylase step (Dickinson and Preiss, 1969) or at the sucrose synthase step (Murata, 1974). At elevated temperature, Macleod and Dufus (1988), Denyer *et al.* (1994) and Jenner (1994) found reduced activity of sucrose synthase in wheat and Lafta and James (1995) in Potato tubers.

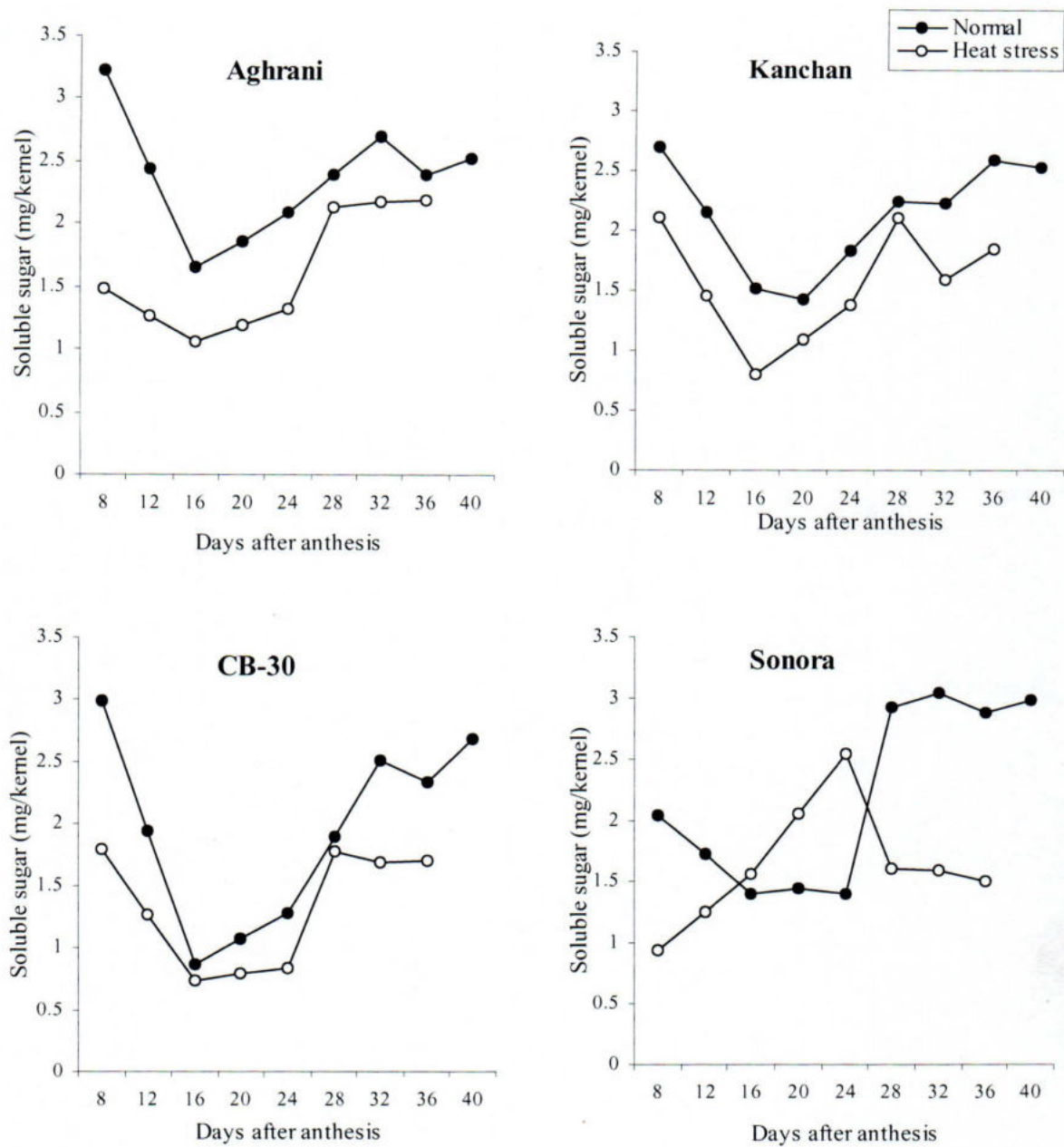


Figure 9. Soluble sugar content in wheat genotypes at different days after anthesis under normal and post anthesis heat stress conditions

4.2.7. Kernel size

Individual kernel size was differed significantly due to the combine effect of growing conditions and wheat genotypes (Figure 10). Under normal growing condition, maximum kernel size was recorded in Aghrani (41.37 mg/kernel) which was statistically equal with that of Kanchan (41.20 mg/kernel). CB-30 obtained the lowest kernel size (38.04 mg/kernel) which followed the kernel size of Sonora (39.41 mg/kernel). These deviations in kernel size among the wheat genotypes were due to their genetic variation. At post anthesis heat stress condition, kernel size reduced significantly in all wheat genotypes. But the magnitude of reduction was different in different genotypes. In this condition, the HS genotype (Sonora) attained lower relative kernel size to normal (77.44%) than the HT genotypes Aghrani (82.94%), Kanchan (84.49%) and CB-30 (91.82 %). Reduced kernel size under heat stress condition might be due to the reduction in rapid kernel growth duration. Results also suggested that reduction in kernel size was also contributed by the impairment of the process involved in sugar to starch conversion due to heat stress.

4.2.8. Height attributes and height of plant

Flag leaf collar height:

Flag leaf collar height was significantly influenced by the interaction effect of growing conditions and genotypes (Table 6). Under normal growing condition, CB-30 attained the highest flag leaf collar height (72.55 cm) which was followed by Kanchan (70.72 cm) and Sonora (68.77 cm). Whereas, Aghrani attained the lowest (64.40 cm) height. These differences in flag leaf collar height among the wheat genotypes were due

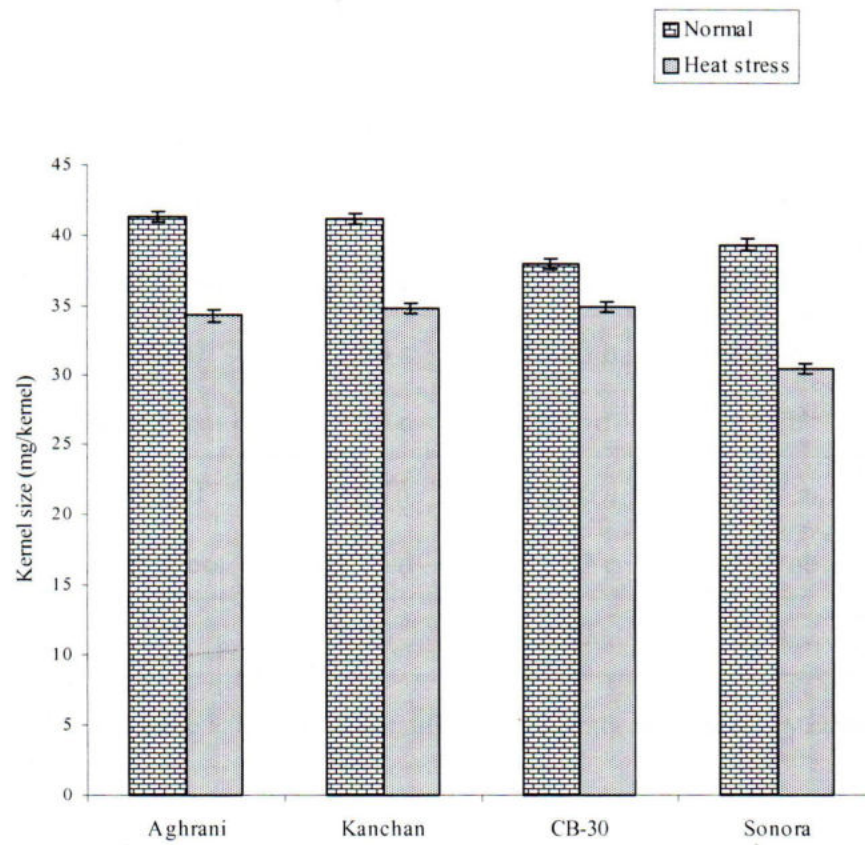


Figure 10. Kernel size of different wheat genotypes under normal and post anthesis heat stress conditions.

Table 6. Height attributes and plant height of f different wheat genotypes under normal condition and post anthesis heat stress conditions

Genotypes	Growing conditions	Flag leaf collar height (cm)	Extrusion length (cm)	Spike length (cm)	Plant height (cm)
Aghrani	Normal	64.40 c	15.22 a	11.66 a	91.28 b
	Heat stress	62.11d	11.39 c	11.15 a	84.65 cd
Kanchan	Normal	70.72 b	14.89 a	11.17 a	96.78 a
	Heat stress	64.79 c	10.93 c	11.17 a	86.89 c
CB-30	Normal	72.56 a	13.43 b	9.64 b	95.63 a
	Heat stress	64.20 c	8.71 d	9.68 b	82.68 d
Sonora	Normal	68.77b	14.36 a	11.37 a	94.50 a
	Heat stress	61.12 d	11.62 c	10.66 ab	83.40 d
CV(%)		4.50	4.63	3.41	2.07

Means followed by the same letter (s) did not differ significantly at %5 level by DMRT

to their varied genetic make up. Under heat stress condition, flag leaf collar height was reduced significantly in all wheat genotypes but the degree of reduction was significantly higher in CB-30 (11.52%) and Sonora (11.12%) than that in Aghrani (3.56%) and Kanchan (8.38%). Reduced flag leaf collar height under heat stress condition might be due to reduced pre-anthesis growth duration. Haque (2002) reported greater reduction in pre-anthesis growth duration in sensitive genotypes than in heat tolerant one.

Extrusion length:

The interaction effect of growing conditions and wheat genotypes on extrusion length was significant (Table 6). Among the wheat genotypes, Aghrani had the longest extrusion (15.22cm) which was statistically similar to that of Kanchan (14.89cm) and Sonora (14.36 cm) but CB-30 had the smallest extrusion (13.43cm) at normal growing condition. Under post anthesis heat stress condition, extrusion length was significantly decreased in all genotypes, though the reduction was higher in CB-30 (35.14%) than that in other wheat genotypes (19.08 to 25.16 %).

Spike length:

Growing conditions did not influence the spike length significantly though it was differed significantly among the wheat genotypes (Table 6). Irrespective of growing conditions. Aghrani, Kanchan and Sonora produced more or less equal spike length (10.66 to 11.66 cm) but it was significantly lower in CB-30 (9.64 to 9.68 cm). Under heat stress condition, the spike length was somewhat reduced in Aghrani and Sonora but in CB-30, it was even increased though the difference was not significant.

Plant height:

The combine effect of growing conditions and genotypes on plant height was significant (Table 6). Under normal growing condition, Kanchan was the tallest (96.78 cm) genotype, which did not differ significantly with CB-30 (95.63 cm) and Sonora (94.50 cm), whereas Aghrani was the smallest (91.28 cm) genotype. This difference in plant height among the wheat genotypes were due to their genetic make up. Under heat stress condition, plant height reduced significantly in all wheat genotypes. The degree of reduction was the lowest in Aghrani (7.26%) which was closely followed by Kanchan (10.22%) and Sonora (11.74%), whereas the highest reduction was found in CB-30 (13.54%).

The reduced plant height under heat stress was due to the reduced flag leaf collar height and extrusion length. Campbell and Read (1968), Bhatta *et al.* (1994), Begga and Rawson (1977) also found shorter plant height due to late sowing.

4.2.9. Spike characters

Number of effective tillers and spikes:

Tiller number per plant and number of spikes m^{-2} were not influenced significantly by the interaction effect of growing conditions and genotypes but its were significantly differed among genotypes (Table 7). Kanchan had the highest tillers per plant (6.5 to 6.65) which was followed by CB-30 (5.25), Sonora (4.70 to 5.00) and it was the lowest in Aghrani (4.58 to 4.68). Again, Kanchan had the highest spikes m^{-2} (273 to 277) which was statistically similar to that of Sonora (260 to 261). Aghrani had the lowest spikes m^{-2} (222 to 225) which was at par with CB-30 (228 to 229).

The insignificant interaction between growing conditions and wheat genotypes indicated that the differences in number of tillers per plant and spikes m^{-2} among the genotypes were mainly genetically controlled and wheat plants did not suffer from heat stress in GS₁ phase (emergence to double ridge) in the present study, in which tillering was determined.

Results from other studies showed that the spike number per plant and spikes m^{-2} were not influenced by late sowing condition (Sikder, 1999). He and Rajaram (1993) reported that the ear number m^{-2} was less sensitive while yield, grains per ear, biomass and plant height were more heat sensitive. Shanahan *et al.* (1990) mentioned that heat tolerant genotypes were associated with productivity only under extreme temperature. These findings partially supported the present finding.

Spikelets per main shoot spike:

Irrespective of growing conditions, Aghrani and Kanchan contained significantly higher spikelets per spike than that of CB-30 and Sonora (Table 7). It might be due to genetic variation among the genotypes. Under post anthesis heat stress condition, this number remained more or less same in Kanchan and CB-30 as under normal condition. But in Aghrani and Sonora, it was reduced 4% and 3.2%, respectively, though the reduction was not significant. The spikelets number per spike was determined during GS₂ phase (double ridge to anthesis) and it was reduced under heat stress (Shpiler and Blum, 1986). In the present experiment, significant reduction was not found. It might be due to the wheat genotypes in this study did not suffer from heat stress in GS₂ phase.

Table 7. Spike characters of different wheat genotypes under normal condition and post anthesis heat stress condition

Genotypes	Growing conditions	No. of effective tillers per plant	No. of spikes per m ²	No. of spikelets per spike	No. of florets per spike	Floret sterility (%)	No. of kernels per spike
Aghrani	Normal	4.68 d	225 b	19.40 a	94.58 a	36.30 c	60.25 a
	Heat stress	4.58 d	222 b	18.60 ab	93.86 a	37.94 bc	58.25 a
Kanchan	Normal	6.65 a	277 a	19.65 a	79.68 c	38.19 abc	49.25 de
	Heat stress	6.15 a	273 a	19.65 a	72.37 d	31.25 abc	47.15 e
CB-30	Normal	5.25 b	229 b	17.85 b	93.60 a	36.43 c	59.50 a
	Heat stress	5.25 b	228 b	17.95b	93.16a	41.55 a	54.45 b
Sonora	Normal	5.00 c	261 a	18.40b	84.33 b	38.04 bc	52.25 bc
	Heat stress	4.70cd	260 a	17.80 b	80.31 c	40.82 ab	47.53 e
CV(%)		3.83	3.53	6.06	6.17	5.70	3.15

Means followed by the same letter (s) did not differ significantly at %5 level by DMRT

Florets per main shoot spike:

Number of florets per spike was varied significantly by the combined effect of growing conditions and wheat genotypes (Table 7). Under normal growing condition Aghrani contained the highest number of florets per spike (94.58) which was statistically similar to that of CB-30 (93.60). Sonora (84.33) followed the CB-30 whereas Kanchan contained significantly the lowest (79.68) florets per spike. Under post anthesis heat stress condition, the florets number per spike was reduced. The reduction was not significant in Aghrani and CB-30 though it was significant in Kanchan and Sonora. Significant reduction in the number of florets per spike in wheat under heat stress was also reported by Shpiler and Blum (1986), Amores *et al.* (1984) and Zeng *et al.* (1985).

Floret sterility:

The floret sterility (%) was influenced significantly by the interaction effect of growing conditions and genotypes (Table 7). All the wheat genotypes showed statistically similar percent sterility (36.30 to 38.19 %) in normal growing condition. Post anthesis heat stress increased the sterility in all wheat genotypes though the increment was significant only for CB-30.

From the results of other studies it was found that high temperature resulted in floret sterility (Rawson, 1986), late planting wheat experienced high temperature at reproductive stage causing abortion of florets (Warrington *et al.*, 1977).

Kernels per spike:

Kernel number per spike was influenced significantly by the interaction effect of growing conditions and genotypes (Table 7). Under normal growing condition, Aghrani had the highest number of kernels per spike (60.25) which was statistically similar to that of CB-30 (59.50) and the Kanchan had the lowest kernels per spike (49.25). Whereas, in case of Sonora, it was the second lowest (52.25). On the other hand, at post anthesis heat stress condition, this number was reduced in all genotypes though the reduction was significant only for CB-30 and Sonora. Based on magnitude of reduction in kernels per spike the HS genotype Sonora affected more (9.3%) than the HT genotypes Aghrani (3.3%), Kanchan (4.3%) and CB-30 (8.5%).

Reduced kernels per spike under heat stress condition was due to the reduced florets per spike and increased floret sterility (%) shown in Table 7. Reduced number of kernels per spike in different magnitude were also observed by Islam *et al.* (1993), He and Rajaram (1993), Al-Khatib and Paulsen (1990) and Bhatta *et al.* (1994) under late sowing or high temperature condition compared to optimum sowing temperature.

4.2.10. Spike weight, yield and heat susceptibility index

Main shoot spike dry weight:

The combine effect of growing conditions and genotypes on spike dry weight was significant (Table 8). At normal growing condition, Sonora had the heaviest spike (3.41 g), which was statistically similar to that of Aghrani (3.35 g). The lowest spike dry weight was obtained in Kanchan (2.64 g), which differed significantly with that of CB-30

(3.04 g). In post anthesis heat stress condition, spike dry weight was reduced in all wheat genotypes though the reduction was insignificant in CB-30.

The HS genotype Sonora showed the lower relative spike dry weight to normal (81.23%) compared to that in HT genotypes such as , in Aghrani (87.76%), Kanchan (88.26%) and CB-30 (99.67%) graphically shown in figure 11. The reduction of spike dry weight under heat stress condition might be due to reduced florets and kernels number/spike (Table 7).

Kernels dry weight per spike:

Significant variation was observed in kernels dry weight per main shoot spike by the interaction effect of growing conditions and genotypes (Table 8). Aghrani obtained the highest kernel dry weight (2.60 g/spike) which was at par with Sonora (2.55 g). It was closely followed by CB-30 (2.33 g) and Kanchan obtained significantly the lowest value (2.00 g) under normal growing condition. Both in HT and HS wheat genotypes the kernel weight was reduced due to post anthesis heat stress condition. But different genotypes showed different degree of responses to high temperature. The HS Sonora attained the lower relative kernel dry weight per spike to normal (80.00%) than the HT genotypes (87.69 to 95.71 %) indicating that HS genotype affected more under heat stress condition shown (Figure 12). Similar result was found by Asana and William (1965).

Kernels to spike dry weight ratio:

Aghrani obtained significantly the highest (0.776) kernels to spike dry weight ratio, which was followed by CB-30 (0.769) and Kanchan (0.760) though these value

were statistically equal, whereas Sonora showed significantly the lowest value (0.747) at normal growing condition. These variations among the genotypes were due to genetic factor. At heat stress condition, this ratio was reduced, but the differences were significant in CB-30 and Sonora, Whereas it was insignificant in Aghrani and Kanchan. The relative ratio to normal was lower in Sonora (96.12%) than the HT genotypes Aghrani (99.58%) Kanchan (99.74%) and CB-30 (97.14%) shown in figure 13. The varied magnitude of reduction in kernel to spike dry weight ratio was due to varied degree of reduction in spike dry weight and kernels dry weight per spike under post anthesis heat stress condition.

Straw yield:

The straw yield was significantly influenced by the interaction effect of growing condition and genotypes (Table 8). Maximum straw yield was recorded from Kanchan (4.81 t/ha) which was statistically similar to that of Aghrani (4.71 t/ha) and Sonora (4.66 t/ha) but it was significantly lower in CB-30 (4.38 t/ha). Genetic variation was the responsible factor for this variation under normal growing condition. On the other hand, under heat stress condition straw yield was reduced significantly in all wheat genotypes. But the degree of reduction was varied in different wheat genotypes. Here the relative straw yield to control was lower in HS genotype (79.82%) than that in the heat tolerant Aghrani (81.70%), Kanchan (89.81 %) and CB-30 (90.18).

The different magnitude of reduction in straw yield among the wheat genotypes might be due to the different degree of reduction in pre anthesis growth duration at high temperature stress. There are reports that (Haque, 2002) the HT genotypes attained less

Table 8. Spike weight, yield and heat susceptibility index of different wheat genotypes under normal and post anthesis heat stress conditions

Genotypes	Growing conditions	Spike dry wt. (g per spike)	Kernel dry wt. (g per spike)	Kernel : spike dry wt.	Straw yield (t/ha)	Grain yield (t/ha)	Biological yield (t/ha)	Heat susceptibility index
Aghrani	Normal	3.35 a	2.60 a	0.776 a	4.71a	4.94 a	9.06 a	0.962
	Heat stress	2.94 b	2.28 b	0.772 a	3.82c	4.34 bc	7.64 c	
Kanchan	Normal	2.64 c	2.00 c	0.760 b	4.81a	4.32 bc	8.61 b	0.902
	Heat stress	2.33 d	1.77 d	0.758 b	4.32b	3.82 de	7.67 c	
CB-30	Normal	3.04 b	2.33 b	0.769 b	4.38b	4.07cd	7.96 c	0.346
	Heat stress	3.03 b	2.23 b	0.747c	3.95 c	3.89 de	7.34 d	
Sonora	Normal	3.41 a	2.55 a	0.747c	4.66a	4.53 b	8.63 b	1.721
	Heat stress	2.77bc	2.04 c	0.728d	3.72 c	3.54e	6.97 d	
CV(%)		5.49	5.54	1.23	5.54	5.98	5.21	-

Means followed by the same letter (s) did not differ significantly at %5 level by DMRT

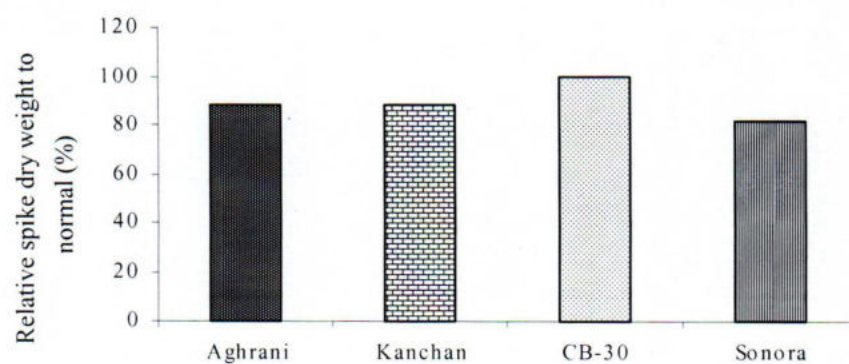


Figure 11. Relative spike dry weight to normal (%) in different wheat genotypes.

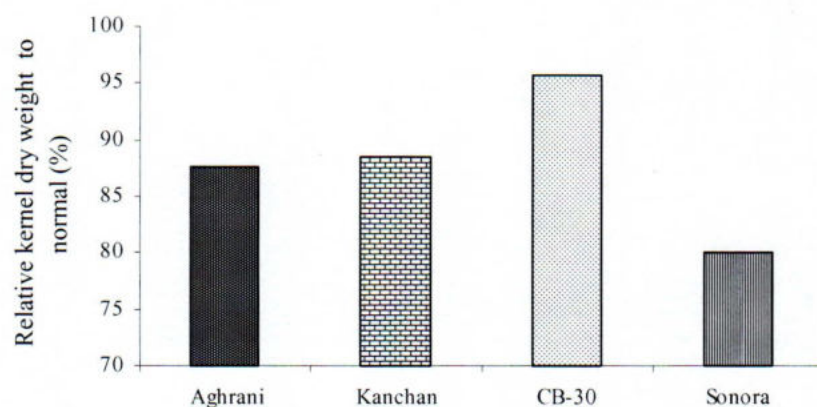


Figure 12. Relative kernel dry weight per spike to normal (%) in different wheat genotypes.

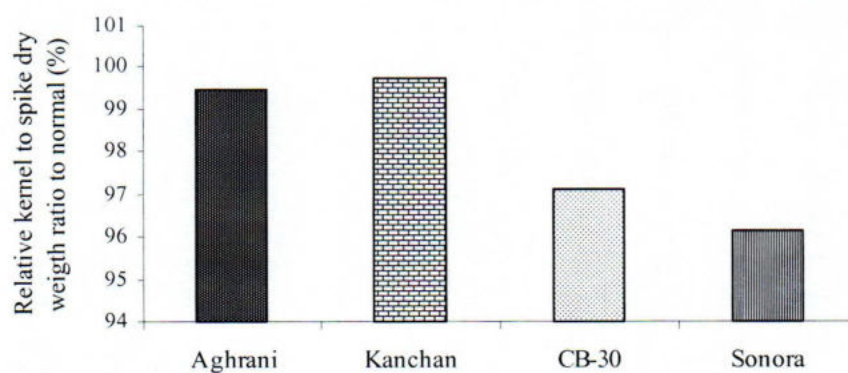


Figure 13. Relative kernel to spike dry weight ratio to normal (%) in different wheat genotypes.

reduction in pre-anthesis growth duration compared to that in HS genotypes under late growing condition. Campbel and Read (1968) also reported less vegetative biomass production under high temperature. These results support the above findings. On the other hand, under heat stress condition straw yield was reduced significantly in all wheat genotypes. But the degree of reduction varied in different wheat genotypes. Here the relative straw yield to control was lower in HS genotype (79.82%) than that in the heat tolerant Aghrani (81.70%), Kanchan (89.81 %) and CB-30 (90.18).

The different magnitude of reduction in straw yield among the wheat genotypes might be due to the different degree of reduction in pre anthesis growth duration at high temperature stress. There are reports that (Haque, 2002) the HT genotypes attained less reduction in pre-anthesis growth duration compared to that in HS genotypes under late growing condition. Campbel and Read (1968) also reported less vegetative biomass production under high temperature. These results support the above findings.

Grain yield:

Growing conditions and wheat genotypes interacted significantly to govern the grain yield (Table 8). Under normal growing condition, Aghrani attained the highest grain yield (4.94 t/ha) which was followed by Sonora (4.53 t/ha) and Kanchan (4.32 t/ha) whereas CB-30 gave the lowest grain yield (4.07 t/ha). Post anthesis heat stress condition decreased the grain yield significantly in all wheat genotypes but the amount of reduction was not equal in different genotypes. Percent relative yield to normal (78.15%) was lower in heat sensitive Sonora and that was higher in the heat tolerant Aghrani (87.80%), Kanchan (88.43%) and CB-30 (95.58%). In the present study it was 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 (Figure 5 and Table 8).

Reduced number of kernels per spike and reduced kernel size were the major responsible factors for reducing the grain yield under heat stress condition in the present experiment. Other yield attributes e.g., spikes number m⁻² was not affected at high temperature (Table 7). Results from other studies showed that late sowing caused lower grain yield in wheat compared to optimum sowing (Islam *et al.*, 1993; Bhatta *et al.*, 1994). Al-Khatib and Paulsen, (1990) concluded the high relative grain yield which was the results of stable and/or long duration of photosynthetic activity at heat stress condition as a selection criterion for heat tolerance of wheat genotypes.

Biological yield:

Biological yield of wheat was significantly influenced by the interaction effect of growing conditions and genotypes (Table 8). Under normal growing condition, Aghrani gave significantly higher biological yield (9.06 t/ha) than that of Sonora (8.63 t/ha) and Kanchan (8.61 t/ha), though the difference in biological yield between Sonora and Kanchan was not significant. CB-30 gave significantly the lowest yield (7.96 t/ha).

Under post anthesis heat stress condition, biological yield decreased significantly in all wheat genotypes but the degree of reduction was not similar in all genotypes. The HT genotypes, Aghrani, Kanchan and CB-30 showed greater percent relative biological yield to normal (84.3 to 92.21%) than the HS genotype Sonora (80.76%). The reduced biological yield in wheat under heat stress was due to the reduced grain and straw yield. Bhatta *et al.* (1994) also reported reduced biological yield under high temperature.

Heat susceptibility index:

Heat susceptibility index based on grain yield varied in different wheat genotypes (Table 8). According to the susceptibility index, CB-30 (0.346) was found as heat tolerant, Aghrani (0.962) and Kanchan (0.90) were moderately tolerant and Sonora (1.721) was found as heat susceptible genotype.

CHAPTER V

SUMMARY AND CONCLUSION

A laboratory experiment (Experiment-I) was conducted in the Crop Botany Laboratory at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur during 15 October to 15 November, 2001 to study the influence of three temperature regimes (15°C , 25°C and 35°C) on seed reserve mobilization in six wheat genotypes. Wheat genotypes Aghrani, Kanchan CB-30, CB-24 and CB-34 were used as study materials. Another experiment (Experiment-II) was conducted in the research farm at Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur during November, 2001 to May, 2002 to study the influence of post anthesis heat stress on physiology of grain in heat tolerant and heat sensitive wheat genotypes. Four wheat genotypes (Aghrani, Kanchan CB-30, and Sonora) based on seed metabolic efficiency determined in the laboratory experiment were selected as study materials. Two temperature regimes were November 30 sowing regarded as normal and December 30 sowing as post anthesis heat anthesis condition.

Speed of germination expressed as rate of germination, co-efficient of germination and germination vigor index was found to be increased with increasing temperature from 15 to 35°C in all wheat genotypes, but heat tolerant ability based on speed of germination was not found.

Shoot length at 5th day was increased from 15°C to 25°C in all genotypes. At 35°C , shoot length increased in Aghrani, Kanchan and CB-30 whereas in Sonora, CB-24

and CB-34, it was even decreased. Root length was also increased with increasing temperature from 15 to 25°C and thereafter decreased at 35°C in all wheat genotypes.

Shoot dry weight increased with increasing temperature in all genotypes except in CB-34 at 35°C. In case of root dry weight, higher value was found at 25°C and it was affected significantly both at 15°C and 35°C in all genotypes.

Generally root growth in relation to length and dry weight was affected more than shoot at 35°C. But Aghrani, Kanchan and CB-30 can be grouped as less affected. On the other hand, Sonora, CB-24 and CB-34 can be grouped as more affected.

The amount of seed reserve lost as respiration increased from 25°C to 35°C in all genotypes, but the increment was higher in Sonora (14.1%) and CB-24 (13.21%), moderate in Kanchan (6.36%), CB-30 (5.22%) and CB-34 (4.95%) and lowest in Aghrani (2.03%). At 15°C, the amount of seed reserve lost as respiration was also increased from that at 25°C in all genotypes except in Aghrani and CB-30. But again the increment was higher in Sonora, CB-24 and CB-34 (2.79 to 8.84%) than that of Kanchan (1.67). In case of Aghani and CB-30, it was even decreased.

Accumulated of dry matter in shoot increased significantly with increasing temperature from 15 to 35°C but accumulation of dry matter in root increased from 15°C to 25°C and decreased thereafter at 35°C.

At 25°C temperature the seed metabolic efficiency was significantly higher than that at 15°C and 35°C in all wheat genotypes. But Sonora, CB-24 and CB-34 had higher efficiency (2.58 to 3.48 g/g) than that of the remaining genotypes (1.99 to 2.09 g/g) at 25°C. Based on reduction of efficiency both at 15°C and 35°C from that of 25°C, Sonora, CB-24 and CB-34 were found more affected than Aghrani, Kanchan and CB-30.

Based on membrane thermostability test, Aghrani, Kanchan and CB-30 took longer time (120 to 150 min.) to exceed 50% membrane leakage and were recognized as heat tolerant (HT) genotypes. Sonora took only 30 min. to attain 50% membrane leakage and was designated as heat sensitive (HS) genotype.

Due to post anthesis heat stress condition Sonora (HS) exhibited larger increase in flag leaf proline (122%) along with larger decrease in kernel proline (47.2%) than the HT genotypes (14.5 to 47.1% in flag leaf proline and 2.5 to 38.5% in kernel proline).

Both under normal and post anthesis heat stress conditions, a typical sigmoidal pattern of dry matter accumulation in kernel was discernible in all wheat genotypes. Post anthesis heat stress condition reduced dry matter accumulation at peak and also days to attain peak in all genotypes though the reduction in dry matter accumulation was less in HT genotypes (6.59 to 9.0%) than that in heat sensitive Sonora (10.25%). Again, the reduction in days required to attain peak was 4 days in the HT genotypes whereas it was 8 days in (HS) Sonora.

Under normal growing condition, all the genotypes maintained a higher growth rate (more than 1 mg/day/kernel) for longer time, apparently 22-30 days. Post anthesis heat stress condition reduced this duration. The HT genotypes possessed this duration about 16-28 days whereas Sonora maintained the higher growth rate only for 10 days.

The starch accumulation coincided well with dry matter accumulation indicating that starch was the major component of kernel dry weight and it was found upto 31 to 74% of the total kernel dry weight.

The soluble sugar content data revealed that the availability of photosynthate became the limiting factor during the active kernel development period in all genotypes

under normal growing condition. The HT genotypes showed the similar phenomenon in post anthesis heat stress condition. But in the heat sensitive genotype, the capacity to metabolize sucrose to starch rather than the availability of assimilate limited starch accumulation i.e., kernel development became sink limited.

Tiller number per plant and number of spikes per m² were not influenced significantly by the interaction effects of growing conditions and genotypes but its were significantly differed among genotypes. Kanchan had the highest tillers per plant (6.15 to 6.65) which was followed by CB-30 (5.25) and Sonora (4.70 to 5.00) and it was the lowest in Aghrani (4.58 to 4.68). Again, Kanchan had the highest spikes per m² (273-277) which was statistically similar to that of Sonora (260-261). Aghrani had the lowest spikes per m² (222-225) which was at par with CB-30 (228-229).

Post anthesis heat stress condition significantly reduced the flag leaf collar height but the degree of reduction was higher in CB-30 (11.52%) and Sonora (11.12%) than that in Aghrani (3.56%) and Kanchan (8.38%).

Extrusion length was significantly reduced by post anthesis heat stress condition though the reduction was higher in CB-30 (35.14%) than that in other genotypes. Growing conditions did not influence spike length. On the other hand, post anthesis heat stress condition significantly reduced the height of plant. The degree of reduction was the lowest in Aghrani (7.26%) and it was followed by Kanchan (10.22%) and Sonora (11.74%) whereas it was the highest in CB-30 (13.54%).

Number of spikelets per main shoot spike was not affected by post anthesis heat stress condition but it reduced the floret number. But the reduction was not significant in Aghrani and CB-30 though it was significant in Kanchan and Sonora.

Post anthesis heat stress increased the floret sterility (%) in all genotypes but the increment was significant only for CB-30. Number of kernels per spike was reduced under heat stress condition and the reduction was greater in heat sensitive Sonora (9.3%) than the HT genotypes (3.3 To 8.5%). Spike dry weight, Kernels dry weight per spike and spike to kernel dry weight ratio were affected by post anthesis heat stress condition. In relation to these parameters Sonora attained lower relative value to normal than the HT genotypes.

Individual kernel size was significantly reduced under heat stress condition. But the HS genotype (Sonora) attained lower percent relative value to control (77.44%) than the HT genotypes Agrhrani (83.94%), Kanchian (84.49%) and CB-30 (91.82%).

Post anthesis heat stress condition significantly reduced the straw yield, grain yield and biological yield. But greater reduction of these parameters were found in Sonora than the heat tolerant genotypes e.g. in Aghrani, Kanchan and CB-30.

According to heat susceptibility index based on grain yield, CB-30 (0.346) was found as heat tolerant, Aghrani (0.962) and Kanchan (0.902) were moderately heat tolerant and Sonora (1.721) was found as susceptible genotypes. In the present study it was found that grain yield reduced by about 2.6 to 5.8% for each 1⁰C 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.

From the results it might be concluded that under heat stressed condition-

1. Development of root and shoot with minimum cost of respiratory carbon was observed in HT genotypes.

2. Shorter grain filling duration mainly because of poor metabolism of soluble sugar to starch rather than availability of soluble sugar was found in HS genotype.
3. Relative changes to normal growing condition in proline level in kernel and flag leaf were less in HT genotypes than HS genotype.
4. In HT genotypes the yield parameters such as kernel number per spike, kernel weight per spike, individual kernel size, and biological yield.
5. Grain yield reduced by about 2.6 to 5.8% in HT genotypes and about 7.2% in heat sensitive Sonora for each 1⁰C rise in average mean air temperature from normal growing condition during anthesis to maturity.

Finally, higher seed reserve translocation efficiency, longer grain filling duration along with least affected metabolism of soluble sugar to starch, higher proline level in kernel and better yield components contributed to heat tolerance in Aghrani, Kanchan and CB-30 compared to Sonora. However, based on heat susceptibility index for yield CB-30 was found as the most tolerant and Sonora was most susceptible genotype to post anthesis heat stress condition.

CHAPTER VI

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APPENDICES

Appendix 1. Physical properties of the initial experimental soil

<u>Soil properties</u>	<u>Observed value</u>
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 the initial experimental soil

<u>Soil properties</u>	<u>Observed value</u>
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

Appendix 3. Meteorological conditions during crop growing period

Month	Duration (days)	*Temperature ($^{\circ}\text{C}$)		*Relative humidity (%)		Total rain fall (mm)	*Evaporation (mm)
		Max.	Min.	At 9 AM	At 2 PM		
December, 2001	1-10	26.06	14.70	83.90	57.90	Nil	1.86
	11-20	25.59	13.43	93.80	58.33	Nil	2.19
	21-rest	26.11	12.23	76.90	50.54	Nil	1.77
January, 2002	1-10	26.04	13.09	75.00	52.50	Nil	2.20
	11-20	26.94	13.62	80.30	51.30	Nil	2.10
	21-rest	23.79	13.22	77.18	61.27	24.4	1.94
February, 2002	1-10	26.98	12.26	73.10	45.60	Nil	3.00
	11-20	29.70	16.00	73.10	46.10	Nil	3.80
	21-rest	30.85	16.95	72.88	48.41	1.0	3.88
March, 2002	1-10	30.69	17.04	74.90	49.90	13.2	4.13
	11-20	33.48	19.26	62.7	46.30	6.40	5.34
	21-rest	31.68	20.71	75.72	56.72	57.40	4.58
April, 2002	1-10	31.12	21.44	79.40	62.40	89.80	4.48
	11-20	32.35	23.56	76.80	59.90	Nil	4.78
	21-rest	32.42	23.44	77.20	63.00	98.20	6.52

* Indicated average of the duration (days)

Source: Plant Physiology Division, BRRI, Gazipur.

Appendix 4. Kernel soluble sugar content (%) in different wheat genotypes at different days after anthesis both under normal and post anthesis heat stress conditions

Genotypes	Growing conditions	Soluble sugar (%) at different days after anthesis								
		8	12	16	20	24	28	32	36	40
Aghrani	Normal	34.21	21.09	7.97	6.54	4.86	5.05	5.15	6.70	4.47
	Heat stress	18.27	10.56	3.11	3.02	3.20	4.76	4.57	4.05	
Kanchan	Normal	37.31	22.46	8.94	5.48	3.5	4.05	4.37	4.44	5.25
	Heat stress	22.64	13.65	2.72	2.98	3.69	5.14	3.89	3.75	
CB-30	Normal	40.62	25.30	2.92	3.25	3.20	4.21	5.05	4.97	5.64
	Heat stress	21.77	15.32	2.72	2.35	2.23	3.24	3.98	3.45	
Sonora	Normal	30.61	20.40	7.87	5.68	4.57	5.03	5.73	4.63	5.73
	Heat stress	18.46	14.25	4.57	4.95	5.34	3.21	3.50	2.24	

Appendix 5. Kernel starch content (%) in different wheat genotypes at different days after anthesis both under normal and post anthesis heat stress conditions

Genotypes	Growing conditions	Starch content (%) at different days after anthesis								
		8	12	16	20	24	28	32	36	40
Aghrani	Normal	38.57	48.26	56.91	62.19	67.34	69.26	72.19	71.17	70.06
	Heat stress	49.70	60.35	73.84	73.25	73.26	73.76	59.98	58.89	
Kanchan	Normal	31.68	46.35	58.43	61.36	65.70	66.84	67.35	68.15	68.31
	Heat stress	51.07	65.31	72.38	73.01	73.44	73.76	60.85	58.20	
CB-30	Normal	45.25	57.24	65.60	65.69	66.09	66.65	67.15	67.20	67.32
	Heat stress	48.26	62.47	72.19	73.21	73.26	67.42	59.69	58.69	
Sonora	Normal	34.30	62.31	73.74	69.74	66.86	68.12	69.19	64.90	65.80
	Heat stress	40.60	58.64	74.03	73.64	72.68	68.54	59.11	58.14	