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

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Abstract

Seedling of 20 wheat genotypes were grown in Phytotron at about 25 and 35° C for measuring membrane injury in per cent and seedling proline content to investigate seedling proline as screening criterion against heat stress. The wheat genotypes (Bijoy, Sufi, Kanchan, Fang 60, BAW 1059, BL 1883, BL 1022, IVT 7, IVT 8, IVT 9, IVT 10 and BAW 917) showing $< 50\%$ membrane injury were grouped as heat tolerant (HT) and the genotypes (Shatabdi, Prodip, BAW 1064, Gourab, Pavon 76, Sonora, Kalyansona and IVT 6) showing $\geq 50\%$ membrane injury were classified as heat sensitive (HS). At high temperature (35° C) the HT genotypes produced more than double (> 200%) proline than that of 25° C but the HS genotypes produced less quantity of proline at 35° C compared to that in HT genotypes. The seedling proline content at 35° C and membrane injury (%) maintained a significant negative correlation ($r = -0.619$**) across the 20 wheat genotypes tested.

Introduction

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

Genotypic variations in proline accumulation have been observed in many studies and attempts were made to correlate its accumulation with tolerance of plants to stress. This apparent correlation between proline accumulation and environmental stress suggests that proline could have a protective function. Recently, Paleg *et al.* (1981) demonstrated that a number of solutes, including proline, protected enzymes, isolated from various tissues, from inactivation by heat. Rapid catabolism of proline upon relief of stress may provide reducing equivalents that support mitochondrial oxidative phosphorylation and the generation of ATP for recovery from stress and repair of stress induced damage (Hare and Cress 1997). Proline accumulation in high temperature has been reported in barley and radish leaves (Chu *et al*. 1974), in tomato floral buds and leaves (Kou *et al*. 1986), 1.5-fold high in mulberry leaves (Chaitanya 2001), in the leaves of *Brassica* vegetables (Takeda *et al*. 1999), in cotton leaves (Ronde *et al*. 2001), in cabbage and Chinese cabbage (Hossain *et al*. 1995), in apple (Park *et al*. 2001) and in flag leaves of wheat (Hasan *et al.* 2007). Under supraoptimal temperature genotypic difference in proline accumulation pattern has also been reported in six cotton cultivars (Ronde *et al*. 2001) and in different cabbage and Chinese

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cabbage varieties (Hossain *et al*. 1995). Hasan *et al.* (2007) reported genotypic difference in change in flag leaf and kernel proline level in heat tolerant and heat sensitive wheat genotypes due to post anthesis heat stress condition. But in wheat, the heat tolerance in relation to seedling proline content was not yet evaluated. Therefore, the objectives of the present study were to evaluate the effect of high temperature stress on proline accumulation in 20 wheat genotypes and to analyse the suitability of proline accumulation as a heat tolerance index of wheat.

Materials and Methods

The experiment was conducted at Crop Botany Laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during July to November, 2006. Twenty wheat genotypes including most of the popular varieties, some advanced lines and some lines from abroad collected from Wheat Research Centre (WRC) of BARI, Dinajpur, Bangladesh were used for the present study.

Seeds of 20 wheat genotypes were germinated in plastic tray (50 × 35 × 8 cm) filled with soil and allowed to grow in Phytotron (NK SYSTEM BIOTRON, Osaka Nippon Medical & Chemical Instrument Co. Ltd. Tokyo, Japan, Model No. LPH-200-RD) with 16 hrs photoperiod, a light intensity of 200 $\mu E/m^2/s$, relative humidity of 90 ± 1 (%) and at about 25^o C for eight days. Of the six sets of seedlings (three replications) three were allowed to grow in the same condition for another 48 hrs for determination of seedling proline content under normal condition. Another three sets of seedlings were exposed to high temperature stress at 35^o C for 48 hrs. The seedlings were then used for measuring membrane injury (%) and also for determination of seedling proline content. Procedure used for measuring membrane injury to high temperature was the same as described by Blum and Ebercon (1981). The data were analyzed in randomized complete block design and the means were separated by DMRT at 5% level.

Leaf segments (0.5 g) from each replication of each genotype were taken for proline estimation. Subsequently proline was estimated following Bates *et al.* (1973). The proline content was determined from a standard curve and calculated on a fresh weight basis as follows:

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

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

Results and Discussion

Membrane injury to high temperature of ten days old seedling varied significantly among 20 wheat genotypes, with the lowest value in IVT 9 and the highest value in Pavon 76 (Fig. 1). The variation in membrane injury (%) indicates a wide variation in heat tolerance among the wheat genotypes. The wheat genotypes which showed equal or greater than 50% membrane injury were: Shatabdi, Prodip, BAW 1064, Gourab, Pavon 76, Sonora, Kalyansona and IVT 6 and were considered as heat sensitive (HS) genotypes. The other 12 wheat genotypes i. e., Bijoy (41.85%), Sufi), Kanchan, Fang 60, BAW 1059, BL 1883, BL 1022, IVT 7, IVT 8, IVT 9, IVT 10 and BAW 917 showed less than 50% membrane injury in membrane thermostability test and were grouped as heat tolerant (HT) genotypes. The grouping of 17 wheat genotypes out of 20 was consistent with the observations of the previous experiment. In the present study, wheat genotypes Bijoy and Sufi laid in heat tolerant group though they were classified as heat sensitive in the previous experiment. On the other hand, IVT 6 was indicated as heat tolerant in the previous experiment though it was grouped as heat sensitive in the present study.

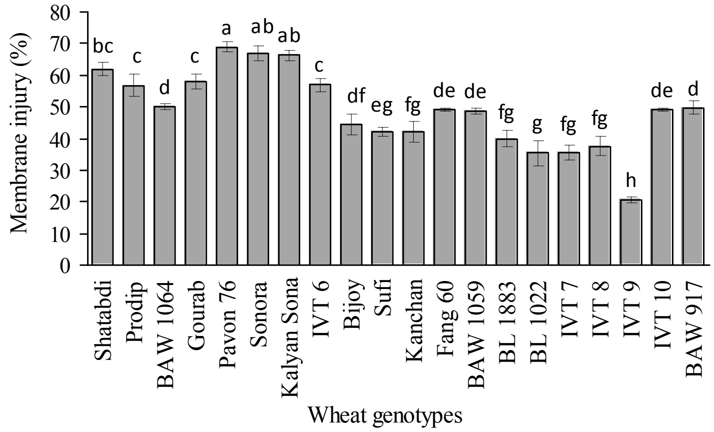

Fig. 1. Membrane injury to high temperature of ten days old seedling of 20 wheat genotypes measured. Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

Proline content of ten days seedlings was influenced significantly by the interaction effect of temperature regimes and wheat genotypes (Table 1). Seedling proline content at 35° C, were higher compared to those at 25° C. The increments of seedling proline content from 25 to 35° C were significant for all wheat genotypes. At 25° C, the seedling proline content was more or less same in heat sensitive and heat tolerant groups, but at 35° C, higher amount of proline was found in HT genotypes comp ... les/g FW) (Fig. 2).

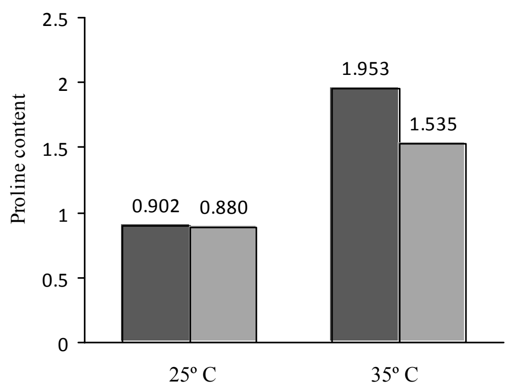

Fig. 2. Average seedling proline content (µmoles/g fresh weight) of heat tolerant and heat sensitive wheat genotypes as influenced by temperature regimes. ■ HT genotypes, ■ HS genotypes.

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

The seedling proline content at 35° C and membrane injury (%) maintained a significant negative correlation (r = – 0.619**) across the 20 wheat genotypes (Fig. 4), indicating wheat genotypes with high proline level at 35° C tended to show greater thermotolerance. This association was insignificant ($r = -0.155^{NS}$) between proline content in seedling at 25° C and per cent membrane injury (Fig. 3).

Table 1. Proline content in ten days old seedling of 20 wheat genotypes as influenced by two temperature regimes. (± SE)

Genotypes	Seedling proline content (µmoles /g fresh weight)		
	At 25^{o} C	At 35^{o} C	Relative to 25^{o} C (%)
Shatabdi	1.13 ± 0.05 f	2.10 ± 0.16 a	186
Prodip	0.82 ± 0.05 gk	1.60 ± 0.05 d	195
BAW 1064	0.87 ± 0.06 gi	1.71 ± 0.07 cd	197
Gourab	0.95 ± 0.06 fi	1.64 ± 0.06 cd	173
Pavon 76	0.60 ± 0.03 k	1.03 ± 0.10 fh	172
Sonora	0.91 ± 0.06 fi	1.36 ± 0.14 e	149
Kalyan Sona	0.73 ± 0.02 ik	1.14 ± 0.06 f	156
IVT 6	1.06 ± 0.06fg	1.70 ± 0.10 cd	160
Bijoy	0.96 ± 0.06 fi	2.08 ± 0.07 ab	217
Sufi	0.64 ± 0.03 jk	1.68 ± 0.05 cd	263
Kanchan	0.85 ± 0.04 gi	1.86 ± 0.05 bc	219
Fang 60	0.80 ± 0.06 hk	1.95 ± 0.05 ab	244
BAW 1059	0.85 ± 0.09 gj	2.12 ± 0.09 a	249
Bl 1883	1.06 ± 0.31 fg	1.50 ± 0.05 de	142
BL 1022	0.98 ± 0.11 fh	1.94 ± 0.02 ab	198
IVT 7	0.92 ± 0.05 fi	1.86 ± 0.05 bc	202
IVT 8	0.91 ± 0.05 fi	2.03 ± 0.04 ab	223
IVT 9	0.87 ± 0.07 gi	2.17 ± 0.14 a	249
IVT 10	1.03 ± 0.07 fh	2.15 ± 0.09 a	209
BAW 917	0.95 ± 0.07 fi	2.09 ± 0.06 ab	220
Mean	0.891	1.786	200
CV (%)	9.42		-

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

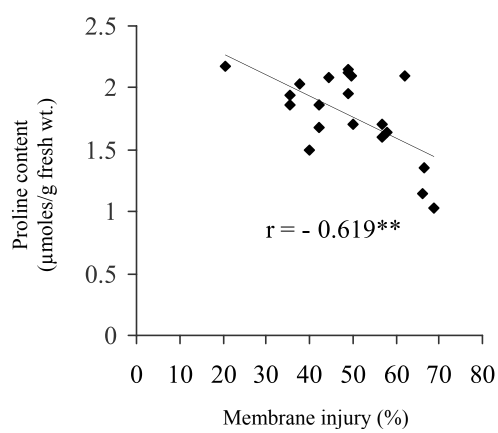

Fig. 3. Relationship ($r = -0.155^{NS}$, n = 20) between membrane injury (%) to high temperature and proline content of seedling at 25^{o} C in 20 wheat genotypes.

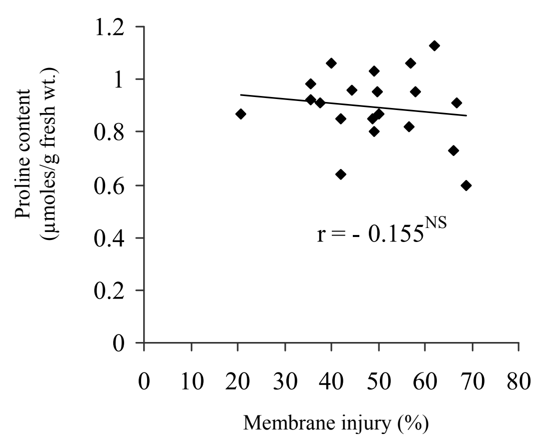

Fig. 4. Relationship ($r = -0.619^{**}$, n = 20) between membrane injury (%) to high temperature and proline content of seedling at 35^{o} C in 20 wheat genotypes.

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

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

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