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Evaluation of Seed Reserve Utilization Efficiency During Germination in Relation to Heat Tolerance of Wheat

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Abstract

Twenty wheat genotypes were tested in a laboratory experiment to investigate seed reserve utilization efficiency (SRUE) during germination as screening criterion against heat stress. The seedling of twenty wheat genotypes were grown on a floating styrofoam stage in water bath for 10 days to measure membrane injury (%) to high temperature. Seeds of those twenty wheat genotypes were also germinated in dark at 25^{o}C and 35^{o}C for 5 days in germinator to determine seed reserve utilization efficiency (SRUE). The wheat genotypes showing <50% membrane injury were grouped as heat tolerant (HT) and the genotypes showing ≥50% membrane injury were classified as heat sensitive (HS) genotypes. At 25^{o}C temperature, all wheat genotypes showed significantly higher seed reserve utilization efficiency (SRUE) during germination (a mean of 1.549 g g^{-1}) as compared to that at 35^{o}C (a mean of 0.880 g g^{-1}). The HT wheat genotypes maintained higher (1.042 g g^{-1}) seed reserve utilization efficiency than that of HS genotypes (0.682 g g^{-1}) at high germination temperature but none of them showed such variation under optimum germination temperature. A significant negative correlation was found across the wheat genotypes between membrane injury (%) and SRUE at 35^{o}C (r = - 0.78**).

Keywords: seed reserve utilization efficiency, heat tolerance, wheat

Introduction

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

A major perturbation to plant productivity under heat stress is ascribed to ‘carbon starvation’ (Levitt, 1982). Heat stress is assumed to impair the plant carbon balance by reducing photosynthetic carbon assimilation and by accelerating carbon loss to respiration. During seed germination the developing wheat seedling is totally dependent on the utilization of the carbon stored in the seed endosperm. Seed reserve utilization for seedling growth in the dark proceeds at an efficiency, which may be calculated as the ratio of the gain in seedling biomass to the loss in seed reserve. Seed reserve utilization for seedling growth in the dark may therefore be considered as a simple model for studying tolerance to heat stress in terms of the carbon balance. In a preliminary study, Hasan et al. (2004) and Cargnin et al. (2006) found that heat tolerant wheat genotypes exhibited lesser loss of seed dry matter than sensitive genotypes during germination. The present study was therefore, conducted to evaluate the hypothesis that heat tolerance in terms of seed reserve utilization efficiency during germination may be associated with the heat tolerance in the autotrophic plant after germination.

Materials and Methods

The experiment was conducted at Crop Botany Laboratory, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur during the period from July to November 2007. 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, Bangladesh were used for the present study.

Membrane Thermal Stability Test

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

$$RI (\%) = (T1/ T2) \times 100$$

where T1 and T2 are the conductivity readings before and after autoclaving, respectively.

Determination of Seed Reserve Utilization Efficiency

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

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

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

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

Where,

SDW = Seed dry weight before germination

SHW = Shoot dry weight

RTW = Root dry weight

RSW = Remaining seed dry weight

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

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

Relative Performance

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

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

Statistical Analysis

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

Results and Discussion

Membrane Injury to High Temperature

Membrane injury (%) of 10 days old seedlings varied significantly among 20 wheat genotypes, with a range of 35.2 to 67.9% and an average of 46.90% (Figure 1). These MI (%) values indicate a wide difference in heat tolerance among the genotypes, as assessed by Ibrahim and Quick (2001). These wheat genotypes were separated into two groups based on membrane injury (%) values. Nine genotypes such as Shatabdi (53.4%), Prodip (51.2%), Bijoy (59.0%), BAW 1064 (60.9%), Sufi (51.5%), Gourab (55.3%), Pavon 76 (67.9), Sonora (53.6%) and Kalyansona (51.9%) showed greater than 50% membrane injury and were classified HS genotypes (an average of 56.08%). The other eleven wheat genotypes i.e., Kanchan (39.2%), Fang 60 (37.8%), BAW 1059 (41.4%), BL 1883 (44.2%), BL 1022 (37.4%), IVT 6 (35.4%), IVT 7 (38.6%), IVT 8 (41.9%), IVT 9 (35.2%), IVT 10 (40.1%) and BAW 917 (42.0%) showed less than 50 MI(%) and were grouped as heat tolerant genotypes (an average of 39.38%).

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

Seed Dry Matter Distribution During Germination

The proportion of seed dry matter accumulation in shoot and root, the amount of dry matter loss as respiration during germination and the dry matter remain in seed at 5 days after placement of germination were significantly influenced by the interaction effect of temperature regimes (25^{o}C and 35^{o}C) and wheat genotypes (Table 1).

The seed dry matter distribution to shoot was significantly higher at 25^{o}C, with a range of 15.4 to 28.5% and a mean of 21.78% compared to that at 35^{o}C (with a range of 5.0 to 9.1% and a mean of 7.17%). But the reduction in shoot dry matter from 25^{o}C to 35^{o}C was more or less same both in HS (from 20.94 to 6.88%) and HT (from 22.48 to 7.41%) wheat genotypes (Figures 2 and 3).

The proportion of seed dry matter translocation to root was significantly higher at 25^{o}C (with a range of 11.5 to 19.9% and a mean of 14.70%) compared to that at 35^{o}C (with a range of 2.3 to 5.6% and a mean of 3.91%). Again the reduction in root dry matter from 25^{o}C to 35^{o}C was more or less

Table 1 Seed dry matter distribution (%) of twenty wheat genotypes (5 days after placement of germination) as influenced by two temperature regimes.

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

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

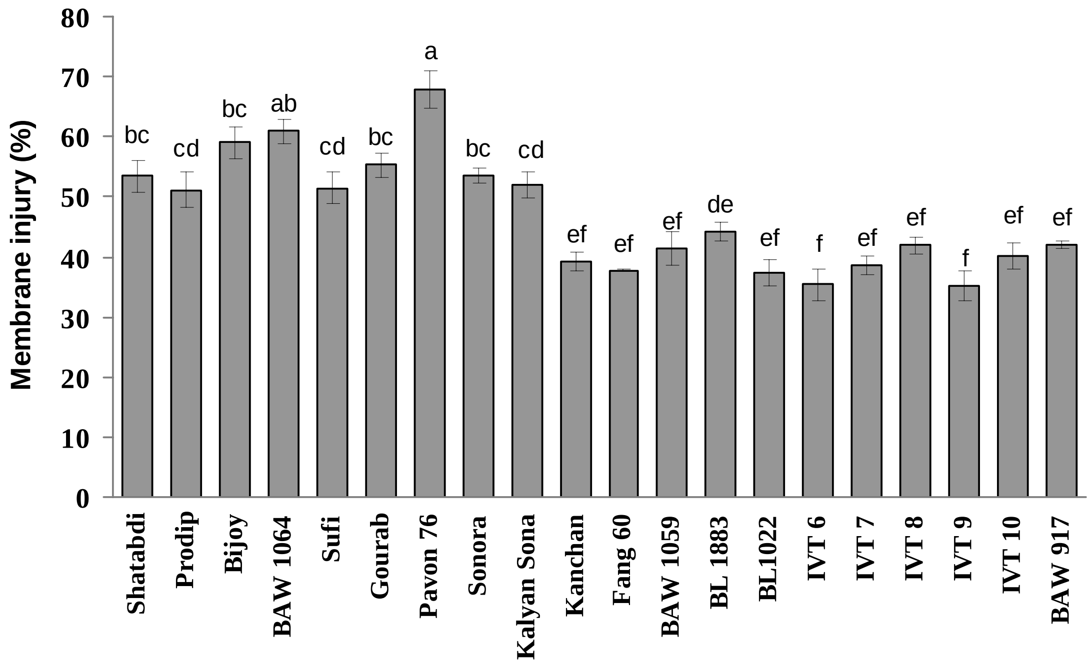

Figure 1 Membrane injury (%) (mean ± SE) to high temperature of 10 days old seedling of 20 wheat genotypes. Values followed by the different letter(s) are significantly different from each other by DMRT at 5% level.

same both in HS (from 14.95 to 3.78%) and HT (from 14.49 to 4.02%) wheat genotypes (Figures 2 and 3).

The amount seed reserve loss due to respiration during germination decreased significantly from 25°C (with a range of 17.1 to 29.3% and a mean of 23.82%) to 35°C (with a range of 8.6 to 20.1% and a mean of 13.26%) in all wheat genotypes. The seed reserve loss at 25°C was more or less same in HS (23.73%) and HT (23.88%) genotypes but at 35°C, higher respiratory loss was found in HS genotypes (15.82%) compare to that in HT genotypes (11.14%) (Figures 2 and 3). At 35°C, the remaining seed contained significantly higher amount of seed reserve (with a range of 67.2 to 81.8% and a mean of 75.69%) compare to that at 25°C (with a range of 29.6 to 50.1% and a mean of 39.59%). The amount of reserve remain in seed at 25°C was more or less same in HS (40.37%) and HT (38.95) genotypes but at 35°C, the remaining seed contained lower amount of dry matter in HS genotypes (73.51%) compare to that in HT (77.43%) genotypes (Figures 2 and 3).

Seed Reserve Utilization Efficiency during Germination

Seed reserve utilization efficiency (SRUE) of dark grown wheat seedlings was significantly influenced by the interaction effect of temperature regimes and wheat genotypes (Table 2). At 25°C temperature, all wheat genotypes showed significantly higher SRUE during germination (with a range of 1.062 to 1.931 g g^{-1} and a mean of 1.549 g g^{-1}) compare to that at 35°C (with a range of 0.507 to 1.348 g g^{-1} and a mean of 0.880 g g^{-1}). The HT wheat genotypes maintained a higher SRUE (1.042 g g^{-1}) than the HS genotypes (0.682 g g^{-1}) at high temperature stress though both groups of the wheat genotypes maintained a more or less same SRUE (1.531 g g^{-1} in HS and 1.563 g g^{-1} in HT genotypes) under normal germination temperature (Figure 4). The reduction in SRUE during germination from 25°C to 35°C is indicated by the relative value. The relative value of SRUE at 35°C compare to 25°C also showed that the reduction in SRUE at high temperature was higher in HS wheat genotypes than in HT genotypes.

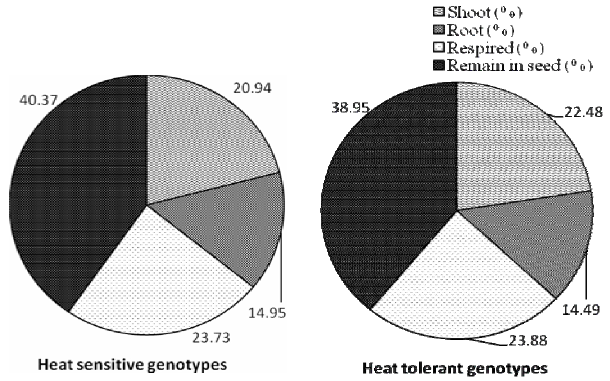

Figure 2 Seed dry matter distribution (5 days after placement of germination) of heat sensitive and heat tolerant wheat genotypes at 25°C.

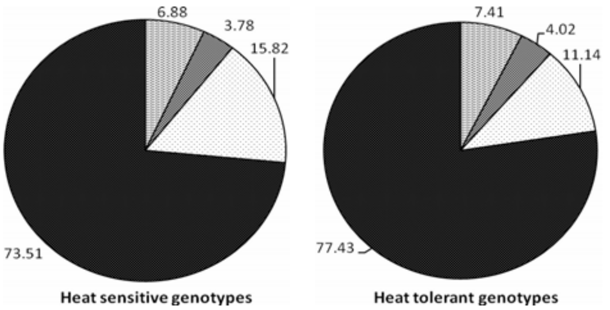

Figure 3 Seed dry matter distribution (5 days after placement of germination) of heat sensitive and heat tolerant wheat genotypes at 35°C.

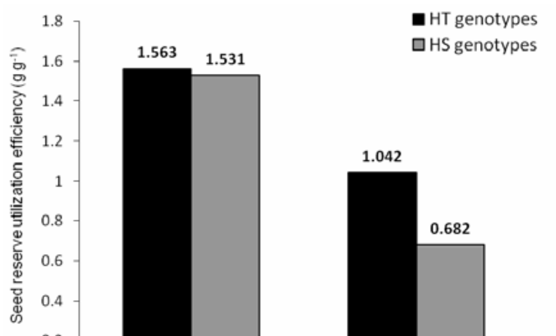

Figure 4 Average seed reserve utilization efficiency (g g^{-1}) of heat tolerant and heat sensitive wheat genotypes as influenced by temperature regimes.

Relationship between Membrane Injury (%) and Seed Reserve Utilization Efficiency

A significant negative correlation (r = -0.78**, n = 20) was found across the wheat genotypes between membrane injury (%) and SRUE at 35°C (Figure 6). Wheat genotypes with low SRUE at 35°C tended to show greater membrane injury.

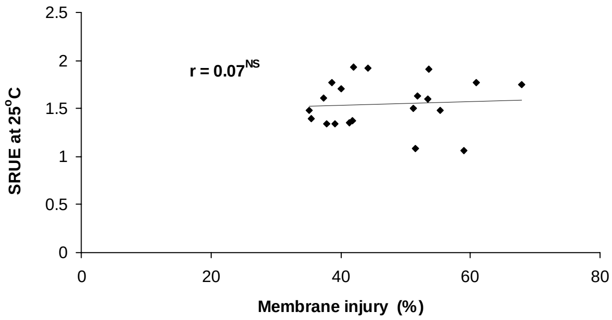

Figure 5 Relationship (r = 0.07^{NS}, n= 20) between membrane injury (%) to high temperature of seedling and seed reserve utilization efficiency during germination at 25°C in twenty wheat genotypes.

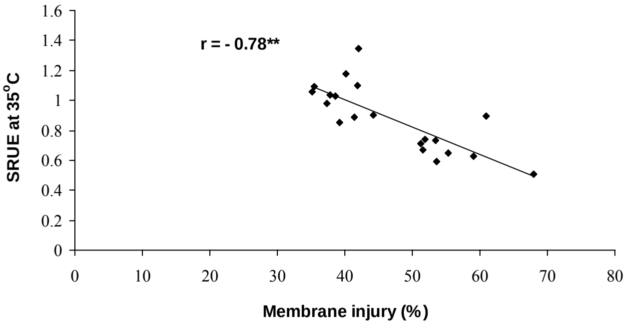

Figure 6 Relationship (r = - 0.780**, n= 20) between membrane injury (%) to high temperature of seedling and seed reserve utilization efficiency during germination at 35°C in twenty wheat genotypes.

This association was insignificant (r = 0.07 NS, n = 20) between membrane injury (%) and SRUE at 25°C (Figure 5).

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

Table 2 Seed reserve utilization efficiency (mean ± SE) of twenty wheat genotypes (5 days after placement of germination) as influenced by temperature regimes.

Genotypes	Seed reserve utilization efficiency (g g^{-1})		
	At 25°C	At 35°C	Relative to 25°C
Shatabdi	1.596±0.05 bc	0.736 ±0.06 jl	0.46
Prodip	1.501±0.01 cd	0.715±0.01 jl	0.48
Bijoy	1.062±0.06 fi	0.629l±0.01 m	0.59
BAW 1064	1.767±0.07 ab	0.896±0.03 gj	0.51
Sufi	1.080±0.03 fh	0.673±0.02 km	0.62
Gourab	1.481±0.05 cd	0.651±0.04 lm	0.44
Pavon 76	1.751±0.14 ab	0.507±0.07 m	0.29
Sonora	1.905±0.08 a	0.595±0.03 lm	0.31
Kalyan Sona	1.636±0.01 bc	0.737±0.03 jl	0.45
Kanchan	1.343±0.05 de	0.854±0.03 ik	0.64
Fang 60	1.336±0.01 de	1.034±0.09 fi	0.77
BAW 1059	1.347±0.03 de	0.885±0.04 hj	0.66
BL 1883	1.917±0.05 a	0.903±0.05 gj	0.47
BL 1022	1.608±0.04 bc	0.978±0.02 fi	0.61
IVT 6	1.398±0.10 d	1.096±0.09 fg	0.78
IVT 7	1.768±0.01 ab	1.029±0.09 fi	0.58
IVT 8	1.370±0.04 de	1.103±0.04 fg	0.81
IVT 9	1.478±0.11 cd	1.057±0.04 fi	0.72
IVT 10	1.701±0.05 b	1.176±0.10 ef	0.69
BAW 917	1.931±0.05 a	1.348±0.16 de	0.70
Mean	1.549	0.880	-
CV(%)	8.86		-

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

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

Conclusions

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

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