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A comparison between synthetic wheat derivatives and bread wheat (*Triticum aestivum*) at seedling stage under various levels of temperature and GA₃ priming

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ABSTRACT

In upcoming years, global warming could produce alarming situation for wheat. Yield and maturity of wheat are critically determined by seedling vigor, with temperature as modifying factor. For the same reason, it is required to determine the adaptation of bread wheat (B) and synthetic wheat derivative (SD) at seedling stage under the influence of temperature fluctuation. The study was conducted to compare synthetic wheat derivative (SD) with bread wheat (B) at low temperature (10°C), moderate temperature (22°C) and high temperature (35°C) with and without gibberellic acid (GA₃) priming at seedling stage. The plant material consisted of four wheat genotypes, two bread and two synthetic wheat derivatives. Following plant parameters were studied viz. germination percentage (G%), germination index (GI), root length (RL), coleoptile length (CL), shoot length (SL) and seed vigor index (SVI). Analysis of variance revealed significant effect of different levels of temperature on wheat genotypes. After making comparison between bread and synthetic wheat derivatives genotypes for all the parameters, results were significantly in favour of SD. Synthetic wheat derivatives obtained relatively higher values of G%, GI and CL at low temperature and G%, GI, SVI, CL, SL and RL at high temperature than bread wheat. High temperature combined with GA₃ priming showed zero results in all genotypes due to GA₃ inactivation at high temperature. These results indicate that synthetic wheat derivatives can perform better relatively than bread wheat at seedling stage, under the influence of temperature fluctuation.

Key Words: Synthetic Wheat Derivative, Bread Wheat, Germination, Gibberellic Acid, Temperature and Genetic Diversity

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I. Introduction

Bread or common wheat (*Triticum aestivum*L.) is a staple food of 4.5 billion people across the globe (Braun et al., 2010). It fulfills 20% of protein and 21% of calories requirement of the people (Braun et al., 2010). Besides having significant global consumption and all the diversity due to three diploid

genomes (A, B and D), bread wheat genome is broad and narrow simultaneously. It is due to low frequency of hybridization between male *Aegilops tauschii* (DD) and female cultivated emmer *Triticum dicoccum* (AABB) naturally. This scenario created lack of genetic diversity of bread wheat due to less exploitation of diverse genome wheats and therefore, it is facing physiological, biochemical and morphological destabilization due to abiotic stresses, especially heat and cold stress with yield loss of 50 kg ha⁻¹ because of increase in 1°C and less germination due to low temperature (Khan, 2004). Keeping consequences in mind, efforts had been made in past decades to develop primary synthetic hexaploids by artificially crossing diploid (DD) and tetraploid (AABB). These primary synthetic hexaploids were utilized to incorporate absent useful genes in modern wheat breeding programs to increase genetic diversity of wheat (Mujeeb-Kazi et al., 1996). As a result, Synthetic wheat derivatives, derived from the primary synthetics and bread wheat crosses, having exceptional resistance to temperature fluctuation (Hughes, 2003).

Seed germination and seedling vigor are critical factors to determine yield and time of maturity, with temperature as modifying factor (Essemine et al., 2007). Due to same reason, good adaptation at seedling stage under the effect of temperature fluctuation could be beneficial for whole upcoming stages of crop. And, eventually it all depends on genetic diversity of genome. Temperature variations could disturb the plant at seedling stage. As, high temperature causes modification in chloroplast structure, organization in thylakoid and eventually photosynthesis (PS II) depletion, due to decrease in biochemical reactions and denaturation of enzymes (Nakamoto and Hiyama, 1999), and finally loss of grain yield (Gibson and Paulsen, 1999). And, low temperature can increase the viscosity of membrane, metabolism damage, and more time consumption in energy dissipation, oxidative stress and radical formation (Beck et al., 2007).

Application of Gibberellic acid (GA₃) on seed, not only at optimum temperature but also at high and low temperature, could unveil the adaptability of synthetic wheat derivatives and bread wheat. As, seed priming with gibberellic acid (GA₃) can cause significant improvement in germination and seedling growth (Ghobadi et al., 2012). As, GA₃ at imbibition stage, stimulate aleurone layer to produce specific RNA molecules encoding alpha-amylase (starch digesting enzyme) through complex interacting network of signaling pathways. It leads to promotion of cambial cell division, germination and hypocotyl growth in wheat (Pospíšilová, 2003). Temperature fluctuation due to global warming could produce alarming situation for wheat in upcoming years. So, the objective of this study is to check the intensity of adaptation of synthetic wheat derivatives and bread wheat by making comparison at different ranges of temperature, along with and without GA₃ priming.

II. Materials and Methods

Experimental Material: The present study was conducted during Aug-Nov, 2019 at plant breeding and genetics laboratory, Faculty of Crops and Food sciences, PMAS- Arid Agriculture University, Rawalpindi. Experimental material consisted of 4 genotypes (2 Bread wheat B1, B2 and 2 Synthetic derivatives SD1, SD2) (Table 01) Synthetic wheat derivatives were obtained by crossing already available synthetic derivatives with respective bread wheat, making common parentage between B1 & SD1 and B2 & SD2. Ten healthy seeds of each genotype were sterilized with sodium hypochlorite to get contamination free seeds, followed by 3 times washing with distilled water. Then seeds were placed in 90-mm-diameter petri dishes on Whatman No.2 filter paper moistened with 10 ml of water (Toklu et al., 2015). Required moisture level (10ml) was maintained by adding the distilled water as and when needed.

Table 01. Pedigree of Bread and Synthetic derivative genotypes

Name Pedigree	
B1	PBW-343
SD1	PBW-343//DOY1/AE.SQUARROSA(188)
B2	INQALAB 91
SD2	INQALAB 91//TSAPKI//SCA/AE.SQUARROSA(518)

B1, bread wheat 1; B2, bread wheat 2; SD1, synthetic derivative 1; SD2, synthetic derivative 2.

Treatments: Comparison of B and SD genotypes for germination percentage were made through six experiments conducted in the incubator (SANYO, MIR-153) available in lab. In first three experiments, petri dishes were placed in incubator at three different temperatures i.e. low temperature (LT=10°C), moderate temperature (MD=22°C) and high temperature (HT=35°C). Next three experiments were done by soaking the sterilized seeds in priming media of GA₃ (500ppm) for 20 hours, then washed thrice with distilled water and dried at room temperature by ventilation to obtain the previous moisture level of seed (Giri and Schillinger, 2003). After drying, petri dishes were placed in the incubator again at three different temperatures (LT, MT and HT).

Estimation of Seedling Parameters:

Seed vigor traits

Germination percentage (G%): Germination count was recorded on daily basis for 5 days after sowing in petri dish. Germination percentage (G%) was calculated by adopting following formula (Ashraf and Abu-Shakra, 1978):

$$G\% = \frac{\text{total germinated seeds}}{\text{total number of seeds}} \times 100$$

Germination Index (GI): Germination index (GI) was calculated by following formula (Association of Official Seed Analysts, 1984):

$$GI \text{ (germination index)} = \frac{\text{number of germinated seeds}}{\text{days of first count}} + \frac{\text{number of germinated seeds}}{\text{days of final count}}$$

Seedling Vigor Index (SVI): Seedling length and germination percentage helped to calculate the seedling vigor index by using the following formula (Kharb et al., 1994):

$$\text{Seedling Vigor Index (SVI)} = \frac{\text{seedling length} \times \text{germination percentage}}{100}$$

Seedling traits

Root length (RL), shoot length (SL) and coleoptile length (CL) were measured with measuring tape and averaged data was obtained by randomly selecting five seedlings of each genotype.

Statistical analysis: Complete Randomized Design (CRD) was used for experiments with three replications. The data for all three replications were averaged to make comparative response of each bread and synthetic wheat derivative genotypes in terms of response to different temperature levels with and without GA₃ priming at seedling stage. Analysis of variance (ANOVA) was done by using Statistix 8.1 computer software.

III. Results and Discussion

Analysis of variance (ANOVA) showed significant effect of different levels of temperature and its combinations with GA₃ priming on all studied parameters viz. G%, GI, SVI, CL, SL and RL in all bread and synthetic wheat derivatives genotypes (Table 02, Table 03).

Comparison of means of bread and synthetic wheat derivatives: Comparison between bread and synthetic wheat derivative showed that SD2 gained highest GI (with GA₃ priming) and SVI (without and with GA₃ priming at moderate temperature). Similarly, highest G% was obtained at moderate temperature by both SD2 and SD1 with and without GA₃ priming, respectively (Table 02). Both types of wheat (bread and synthetic wheat derivative) showed no germination at high temperature combined with GA₃ priming. At low temperature combined with GA₃ priming, both B1 and B2 showed minimum values of G%, GI and SVI (Table 02). SD1 gained longest SL at high temperature (with and without GA₃ priming) and moderate temperature (with GA₃ priming). SD2 obtained longest RL at moderate temperature (without GA₃ priming). And, longest CL was obtained in SD1 and SD2 at moderate temperature with and without GA₃ priming, respectively (Table 03). Bread wheat genotypes showed shortest CL, SL and RL at low temperature with GA₃ priming (Table 03). However, it was

observed that synthetic wheat derivative genotypes performed better than bread wheat genotypes under different conditions (Table 02, Table 03).

Comparison between B1 & SD1 and B2 & SD2: B1 & SD1 and B2 & SD2 share the parentage as mentioned in Table 01. Due to this reason, similarity in trend of increasing and decreasing of all parameters was observed. Therefore, comparison between B1 & SD1 and B2 & SD2 at different conditions is more reasonable. SD1 showed significant highest G% and GI at low temperature with GA₃ priming than corresponding B1 (Figure 01 and Figure 02). SD2 showed significant highest G% and SVI at high temperature (without GA₃ priming) and moderate temperature (with GA₃ priming) and highest GI at moderate temperature (with GA₃ priming), then corresponding B2 (Figure 01, Figure 02 and Figure 06). SD1 gained noteworthy CL at low temperature (without GA₃ priming), SL and RL at high temperature (without GA₃ priming) than corresponding B1 (Figure 03, Figure 04 and Figure 05). SD2 obtained substantial RL at high temperature (without GA₃ priming) and moderate temperature (with GA₃ priming), then corresponding B2 (Figure 05). However, it was observed that difference of efficiency between bread and synthetic wheat derivative genotypes at low temperature (with and without GA₃ priming) was not as significant as compared to other conditions (Figure 01-06).

Table 02. ANOVA showing mean square values of Bread and synthetic derivate cultivars for Germination index and Germination Percentage

SOV	Df	GI %				G %			
		B1	SD1	B2	SD2	B1	SD1	B2	SD2
Temp	2	7400**	6650**	3150**	4838.8**	60.3**	36.5**	5.7**	70**
PGR	1	5000**	2450**	6050**	5688.8**	44.6**	23.2**	123**	17**
Temp X PGR	2	1400**	950**	50**	2038.8**	83.5**	111.1**	32**	116**
Error	10	56.67	70	16.67	32.22	0.07	0.09	0.02	0.02
CV		6.6	7.9	9.3	8.6	5.2	7.4	6.1	5.5

** and * denoted significance at the 0.01 and 0.05 probability level, respectively. CV, coefficient of variation; PGR, Plant growth regulator (GA₃, 500ppm); Temp, temperature; B, bread wheat; SD, synthetic derivative; G%, germination percentage; GI, germination index.

Table 03. ANOVA showing mean square values of Bread and synthetic derivate cultivars for shoot length and coleoptile length

SOV	Df	SL				CL			
		B1	SD1	B2	SD2	B1	SD1	B2	SD2
Temp	2	59.7**	52.3**	48.7**	83.5**	10.5**	10.9**	8.2**	18**
PGR	1	38.7**	51**	79.3**	58.3**	10.1**	5.7**	5.4**	5.7**
Temp X PGR	2	56.4**	132.1**	42.9**	47.5**	3.3**	5.5**	2.2**	2.7**
Error	10	0.09	0.08	0.06	0.07	0.07	0.07	0.07	0.07
CV		6.6	7.9	9.3	8.6	5.2	7.4	6.1	5.5

** and * denoted significance at the 0.01 and 0.05 probability level, respectively. CV, coefficient of variation; PGR, Plant growth regulator (GA₃, 500ppm); Temp, temperature; B, bread wheat; SD, synthetic derivative; SL, shoot length; CL, coleoptile length.

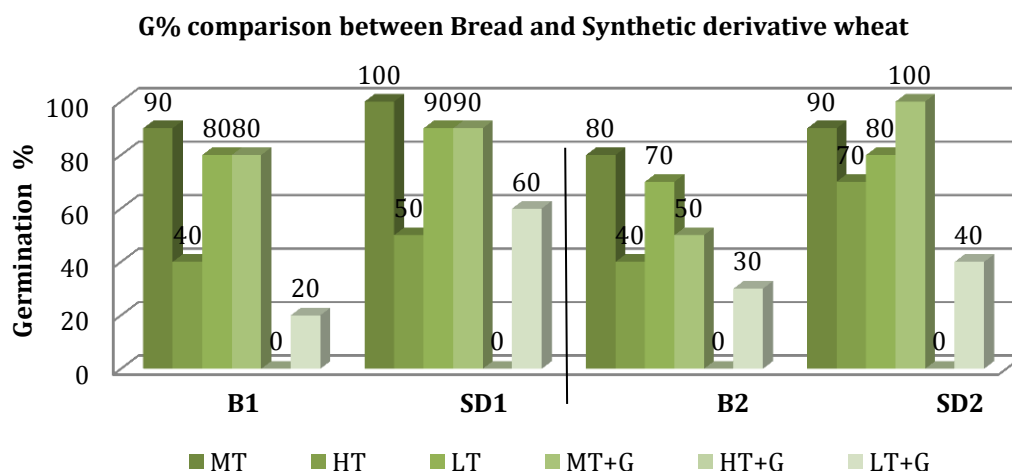
Table 04. ANOVA showing mean square values of Bread and synthetic derivate cultivars for Root Length and Seed vigour Index

SOV	Df	RL				SVI			
		B1	SD1	B2	SD2	B1	SD1	B2	SD2
Temp	2	7.8**	8.8**	20.8**	22.4**	137.6**	148.9**	93.8**	227**
PGR	1	41.4**	69.6**	29.6**	59.4**	49.3**	82.3**	124**	159**
Temp X PGR	2	2.01**	4.08**	8.01**	8.06**	15.9**	40**	28**	34**
Error	10	0.1	0.01	0.02	0.08	0.4	1.01	0.4	0.8
CV		6.6	7.9	9.3	8.6	5.2	7.4	6.1	5.5

** and * denoted significance at the 0.01 and 0.05 probability level, respectively. CV, coefficient of variation; PGR, Plant growth regulator (GA₃, 500ppm); Temp, temperature; B, bread wheat; SD, synthetic derivative; RL, root length; SVI, seed vigor index.

Spring wheat is grown in wide range of environments, exposing to range of temperature fluctuations such as February second half (25/10°C), March (30/15°C) and April (40/25°C). These conditions affect wheat yield negatively at different temperature and result in huge yield losses. Such losses can be compensated by exploiting novel genetic resources. Synthetic wheat derivatives appeared as barrier against temperature fluctuations, due to presence of useful abiotic stress resistant genes (Hughes, 2003). Therefore, critical comparison between bread and synthetic wheat derivative genotypes at seedling stage is a nice strategy to explore genetic resources harboring tolerance to abiotic stresses in wheat (Toklu et al., 2015).

It was observed that without GA₃ priming, G% decreased under both low temperature and high temperature. But the decrease was more under high temperature than low temperature (Figure 01) (Toklu et al., 2015). Without GA₃ priming, high temperature caused increase in GI than moderate and low temperature (Figure 02). It is because; high temperature causes increase in activity of alpha amylase which increases mobilization of seed reserves (Hasan et al., 2004; Sultana et al., 2015). A variable trend of G% was observed with GA₃ priming, for both bread and synthetic wheat derivatives (Figure 01) (Yari et al., 2010). GI after GA₃ priming increases at moderate temperature in both types of wheat (Figure 02). GA₃ priming increases the metabolic activity in seeds which cause enhance in germination speed and synchronization (Sikder and Paul, 2010). However, zero or low efficiency of all parameters (G%, GI, SVI, CL, SL and RL) at high and low temperature with GA₃ priming (Figure 01-06), is due to rapid decrease of incorporation of amino acid (¹⁴C-amino-acid) by GA₃ above 25°C and below 10-15°C, and due to increase in inhibitors at high temperature which effect plant growth (Stoddart et al., 1978; Gonai et al., 2004). RL decreased significantly at moderate temperature with GA₃ priming (Figure 05) (Ghobadi et al., 2012). RL without GA₃ priming, showed efficient increase from low temperature to moderate temperature and decreased at high temperature (Figure 05). It is due to increase in dry matter of root from low to moderate temperature and decrease above moderate temperature (Hasan et al., 2004; Sikder and Paul, 2010). However, large root system plays vital role in valuable traits such as deep-water capture and increase in absorption efficiency (Reynolds et al., 2006).



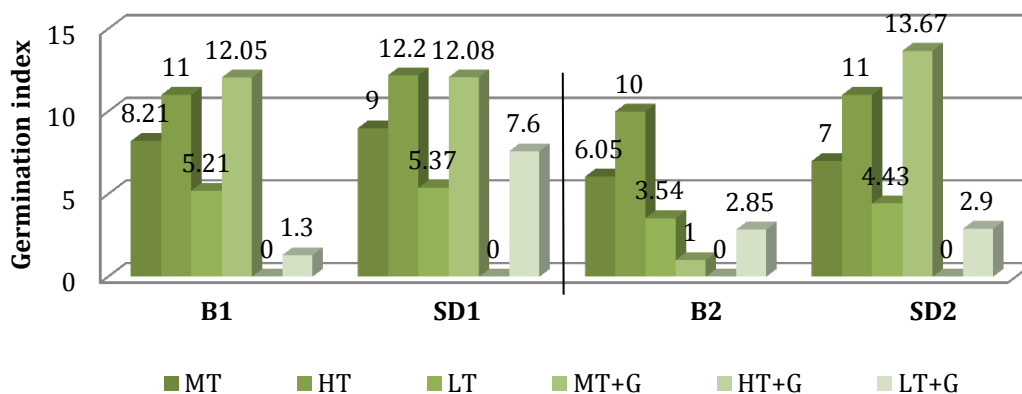
G%, germination percentage; MT, moderate temperature (22°C); HT, high temperature (35°C); LT, low temperature (10°C); MT+G, moderate temperature with GA₃ priming; HT+G, high temperature with GA₃ priming; LT+G, low temperature with GA₃ priming; B, bread wheat; SD, synthetic derivative.

Figure 01. Comparison between G% mean values of two bread and two synthetic derivatives wheat at different temperatures and GA3 priming.

It was observed that first pair of genotypes (B1 and SD1) followed different trend as compared to second pair (B2 and SD2) for all parameters. First pair (B1 and SD1) without GA₃ priming showed more decrease in CL at high temperature than low temperature and long SL at high temperature than moderate temperature and low temperature. Similarly, first pair with GA₃ priming showed long CL and SL at moderate temperature. This trend of first pair (B1 and SD1) is opposite to second pair (B2 and SD2) (Figure 03 and Figure 04). Overall, it was observed that synthetic wheat derivative genotypes originated from same parents of bread wheat genotypes, appeared to be more efficient and consistent at different environmental conditions. However, difference in trend of CL and SL among both types of wheat may be due to the presence or absence of height reducing genes (*Rht1* and *Rht2*). These genes

reduced length CL and SL before GA₃ priming due to insensitivity of endogenous gibberellin and after GA₃ priming due to insensitivity of exogenous gibberellin. So, eventually CL and SL depend on genotype (Rebetzke et al., 1999). However, high CL plays key role in seedling emergence from deeper soils in wheat (Matsui et al., 2002). Highest SVI was obtained at optimum temperature. However, for all environmental conditions variable trends were obtained for SVI (Figure 06). The reason may be that SVI is a product of variable values of seedling (CL, SL and RL) and germination (G% and GI) parameters (ISTA, 2014) and solely depends on these parameters at different conditions.

GI comparison between Bread and Synthetic derivative wheat

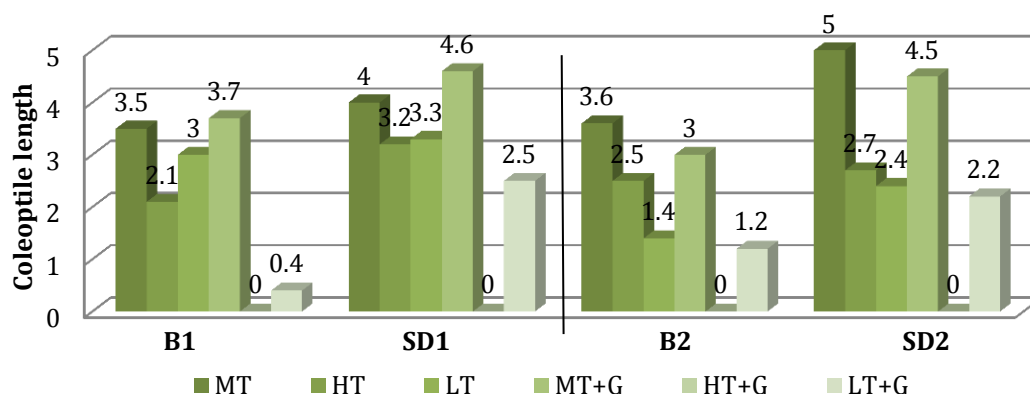


GI, germination index; MT, moderate temperature (22°C); HT, high temperature (35°C); LT, low temperature (10°C); MT+G, moderate temperature with GA₃ priming; HT+G, high temperature with GA₃ priming; LT+G, low temperature with GA₃ priming; B, bread wheat; SD, synthetic derivative.

Figure 02. Comparisons between GI mean values of two bread and two synthetic derivatives wheat at different temperatures and GA₃ priming.

Plants respond to harsh conditions by making changes in their architecture, cell enlargement or due to activation of cold acclimation or thermo-tolerance genes (Halliday and Whitelam, 2003; Penfield, 2008). And, these indicate plasticity in plant’s adaptation to severe conditions (Casal et al., 2004). In our case synthetic wheat derivative genotypes appeared to be more adoptable than bread wheat adjustments genotypes in all optimum and stress conditions. However, absence of significant difference at low temperature between bread and synthetic derivatives for almost all parameters (G%, GI, SVI, CL, SL and RL) was observed, as these limitations were also found for specific environments (Zaharieva et al., 2001).

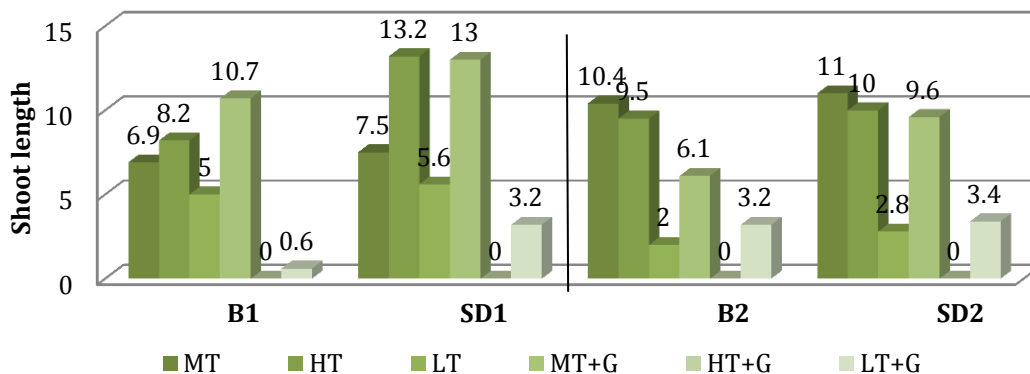
CL comparison between Bread and Synthetic derivative wheat



CL, coleoptile length; MT, moderate temperature (22°C); HT, high temperature (35°C); LT, low temperature (10°C); MT+G, moderate temperature with GA₃ priming; HT+G, high temperature with GA₃ priming; LT+G, low temperature with GA₃ priming; B, bread wheat; SD, synthetic derivative.

Figure 03. Comparison between CL mean values of two bread and two synthetic derivatives wheat at different temperatures and GA₃ priming.

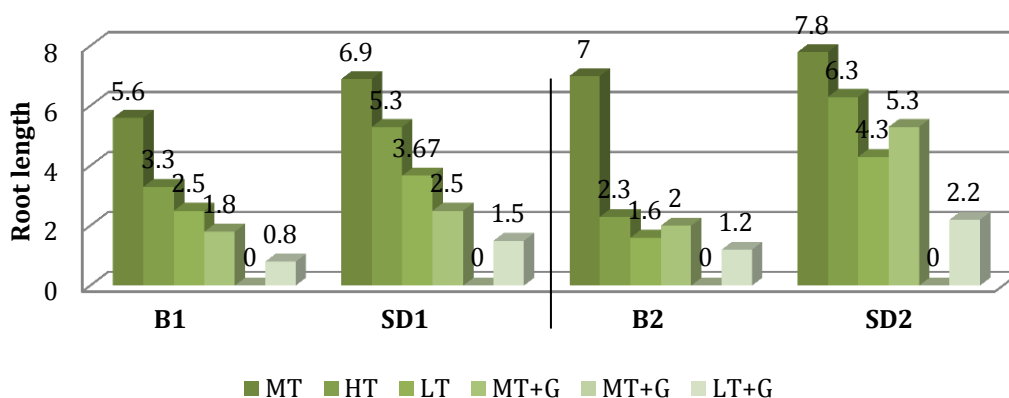
SL comparison between Bread and Synthetic derivative wheat



SL, shoot length; MT, moderate temperature (22°C); HT, high temperature (35°C); LT, low temperature (10°C); MT+G, moderate temperature with GA₃ priming; HT+G, high temperature with GA₃ priming; LT+G, low temperature with GA₃ priming; B, bread wheat; SD, synthetic derivative.

Figure 04. Comparison between SL mean values of two bread and two synthetic derivatives wheat at different temperatures and GA₃ priming.

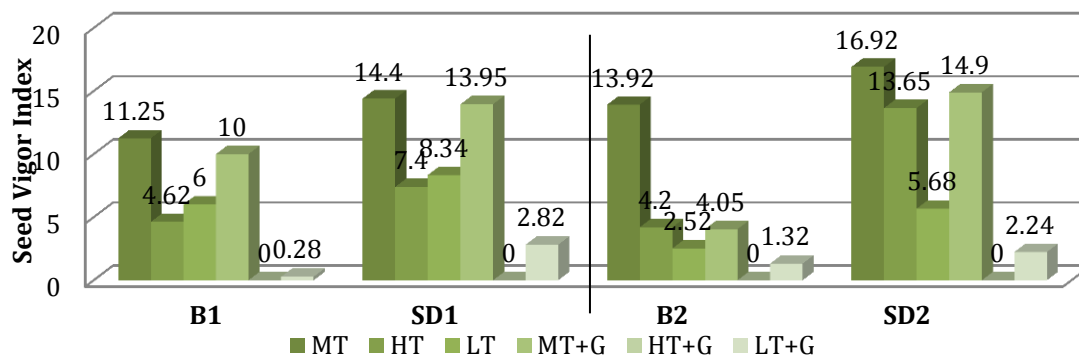
RL comparison between Bread and Synthetic derivative wheat



RL, root length; MT, moderate temperature (22°C); HT, high temperature (35°C); LT, low temperature (10°C); MT+G, moderate temperature with GA₃ priming; HT+G, high temperature with GA₃ priming; LT+G, low temperature with GA₃ priming; B, bread wheat; SD, synthetic derivative.

Figure 05. Comparison between RL mean values of two bread and two synthetic derivatives wheat at different temperatures and GA₃ priming.

SVI comparison between Bread and Synthetic derivative wheat



SVI, seed vigor index; MT, moderate temperature (22°C); HT, high temperature (35°C); LT, low temperature (10°C); MT+G, moderate temperature with GA₃ priming; HT+G, high temperature with GA₃ priming; LT+G, low temperature with GA₃ priming; B, bread wheat; SD, synthetic derivative.

Figure 06. Comparison between SVI mean values of two bread and two synthetic derivatives wheat at different temperatures and GA₃ priming.

IV. Conclusion

In conclusion, synthetic wheat derivative genotypes can be effectively substitute bread wheat genotypes against temperature stresses and certain limitations at low temperature can be overcome by exploiting cold resistant genes from diploid and tetraploid wheat.

V. References

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