

MITIGATING THE ADVERSE EFFECTS OF DROUGHT STRESS THROUGH SEED PRIMING AND SEED QUALITY ON WHEAT (*Triticum aestivum* L.) PRODUCTIVITY

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Drought stress, at reproductive phase in particular, severely reduces wheat yield but some agronomic techniques have the potential to overcome this damage. A field experiment was planned to evaluate the role of seed priming and seed size in drought tolerance of wheat. Bold (1000-seed weight 44.4 g) and small (1000-seed weight 22.4 g) sized seeds were soaked in CaCl₂ solution (osmopriming; ψ_s -1.25 MPa) while untreated dry seeds were taken as control. Crop was grown by using primed and untreated seeds under well-watered conditions till booting stage and then put under drought stress (50% of the field capacity) or control (100% of the field capacity) till biological maturity. Drought stress substantially decreased the wheat productivity due to significant reduction in allometric i.e. crop growth rate (CGR), leaf area index (LAI) and yield components. Moreover, osmoprimed seeds with CaCl₂ over dry seeds and large sized seeds over small sized seeds maintained their dominance due to early and uniform stand establishment, improving crop allometry and productivity due to notable expansion in entire yield related traits under well-watered and drought stress conditions. In conclusion, osmoprimed bold sized seeds may have a vital role in improving wheat production by early and uniform stand establishment, improving crop allometry and productivity due to considerable expansion in yield related traits under normal irrigated and drought stress conditions.

Keywords: Drought stress, osmo-priming, productivity, seed priming, seed size

INTRODUCTION

Food security is becoming an emerging global issue with increase in world population. The world population will reach almost 9 billion up to 2050 (UNPD, 2006). According to estimation, 1.5% annual rise in agricultural productivity should be needed to feed this rising population (FAO, 2006; UNPD, 2006). Wheat (*Triticum aestivum* L.) has a central role in world food security; as it represents more than 1/4th of the global total grain production which serves as a major source of staple food for over 1/5th of human population worldwide (FAO, 2011).

Now a day drought is the single most damaging constraint lowering crop production worldwide (Farooq *et al.*, 2012, 2014; Hussain *et al.*, 2018). This situation becomes more severe in those regions where supplementary irrigation is an unavoidable practice to crop production. Although the wheat plants need adequate water supply throughout their life cycle for healthy growth but there are certain growth phases which are very critical and any shortage on these phenophases paid heavy yield tax (Madani *et al.*, 2010; Farooq *et al.*, 2014, 2015; Hussain *et al.*, 2016). Wheat crop needs more water when it reaches at reproductive and grain-filling phases and deficiency at this stage definitely lower down grain yield more seriously (Nawaz *et al.*, 2013; Farooq *et al.*, 2014). At

the start of crop cycle, drought is less destructive (Blum, 1996) but this situation become severe as the time passed and mild drought at post-anthesis stage may cause reduction in 1-30% and severe stress at reproductive and early maturity stage may reduce final yield up to 90% (Dhanda and Sethi, 2002; Eskandari and Kazemi, 2010; Farooq *et al.*, 2014). Declined grain count due to pollen abortion and grain size due to decreased grain-filling rate and duration are the major motives of terminal drought induced yield loss in wheat (Madani *et al.*, 2010; Wei *et al.*, 2010; Farooq *et al.*, 2014). Nonetheless, last week of October to early November is the ideal time for wheat cultivation in Pakistan but due to late maturing varieties of Basmati rice and late picking of cotton compelled the wheat cultivation up to late December. However, any delay in wheat planting after mid November resulted in heavy yield loss at 60 kg ha⁻¹ per day (Hussain *et al.*, 2012a, b). The chief reason behind this yield penalty is the suboptimal plant population due to erratic and poor germination owing to low temperature existing during late December (Hussain *et al.*, 2012a, b). There are some agronomic techniques like seed priming and use of large seeded size which have the potential to improve the wheat production under optimal and less than optimal growing environment (Dohuki *et al.*, 2011; Farooq *et al.*, 2015; Haider *et al.*, 2016; Nawaz *et al.*, 2017).

Improved germination rate and synchronization allow better growth of primed seeds which results in healthy crop stands even under less than optimal conditions (Hussain *et al.*, 2013, 2017a, b). Recently Farooq *et al.* (2015) reported the better performance of bread wheat subjected to osmopriming with CaCl_2 under well-watered and terminal drought stress conditions. Likewise, many reports available in published literature which concluded that wheat crop emerged from bold seeds produced early and healthy seedlings with more root length and dry matter, and ultimately resulted in higher grain yield due to noteworthy expansion in grain size and count than the crop emerged from small seeds (Robert and Stogarda, 2004; Dohuki *et al.*, 2011; Haider *et al.*, 2016). Although there are lot of studies that investigated the role of priming and seed size in the improvement of wheat production but to best of our reviewed literature, no study has been done to investigate the interactive effects of osmopriming and seed size on wheat productivity under terminal water stress. Therefore, current project was planned on the hypothesis that osmoprimed bold size seeds have the potential to improve wheat performance subjected to terminal drought stress.

MATERIALS AND METHODS

Experimental site: A field experiment was conducted at Agronomic Research Farm, Department of Agronomy, Bahauddin Zakariya University, Multan (71.43°E, 30.2°N), Pakistan. Soil used for this trial was silty clay and a little saline in nature. Fertility status of soil was checked before the sowing of crop. The proportion of sand, silt and clay were 24.64, 53.00 and 20.36%, respectively. Physio-chemical analysis of soil indicated the pH (8.34), EC (3.35 dS m^{-1}), organic matter (0.57%), total N (0.029 ppm), total P (8.79 ppm) and total K (193 ppm). Important meteorological aspects of experimental site are presented in Table 1.

Experimental details: Wheat variety Punjab-2011 was used as experimental material and its seed was taken from Punjab Seed Corporation. Seed lot was separated in two sized seeds i.e. bold (1000-seed weight; 44.4 g) and small sized (1000-seed weight; 22.4 g) seeds. For osmopriming, seed were soaked for 24 hours in the solution of CaCl_2 (-1.25 MPa). After 24 hours seeds were removed from the solution and washed with tap water and placed for drying under shade until the seed gained their original weight while untreated (dry) seeds were taken as control. Crop was grown under well-

watered conditions till booting stage and then induced the drought stress by maintaining the 50% field capacity till physiological maturity and 100% field capacity were maintained for well-watered treatment. According to Feeke's scale these are 10.1 to 11.4 (Large 1954). The field capacity was maintained on gravimetric basis (Nachabe, 1998). Experiment was arranged in RCBD with split-split plot arrangement by keeping drought stress, seed size and priming techniques in main, sub and sub-sub plots, respectively. All experimental units were replicated four times with net plot size of 5 m \times 2.5 m.

Crop husbandry: First irrigation (10 cm) was done for seedbed preparation to produce the favorable condition. When soil reached to *wattar* condition, soil was prepared according to standard agronomic protocol. Wheat was cultivated on 24 November, 2012 with hand drill with seed rate @ 125 kg ha^{-1} in 25 cm spaced rows. Nitrogen (N) and phosphorus (P) fertilizer were applied @ 150 and 100 kg ha^{-1} respectively by using triple super phosphate and urea as source. Half of the N and whole P were applied at the time of sowing and remaining N was side dressed with 1st irrigation. Field was irrigated to maintain well-watered conditions and all other standard protocols of plant protection and plant production were followed. Mature crop was harvested on April 14, 2013.

Observations recorded: For stand establishment parameters, seed germination was counted by following the Handbook of Association of Official Seed Analysts (1990). Days to emergence, mean emergence time (MET) (Ellis and Robert, 1981) and time to 50% emergence (E_{50}) was calculated by using the standard procedure adapted by Coolbear *et al.* (1984) and modified by Farooq *et al.*, (2005).

For the allometric traits like LAI, LAD, and CGR crop sampling were taken after twenty days interval. Leaf area was computed with leaf area meter (DT Area Meter, Model MK2, Delta T Devices, Cambridge, UK). Leaf area per unit ground area was taken as LAI. Protocol given by Hunt (1978) was used to calculate LAD. An area of 0.5 m^2 was selected randomly from each plot after 20 days interval for dry matter accumulation which was then used to calculate crop growth rate as proposed by Hunt (1978).

$$\text{CGR} = (w_2 - w_1) / (t_2 - t_1)$$

Where, W_1 and W_2 represent dry weight of spikes at 1st and 2nd harvest, and T_1 and T_2 represents the days at 1st and 2nd harvest.

All agronomic traits, yield and yield components, measured,

Table 1. Weather data during the course of study.

Weather element	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.
Mean temperature (°C)	19.9	14.8	12.3	16.0	22.0	26.9
Average Relative humidity (%)	84.1	83.5	80.4	87.3	76.1	60.9
Sunshine (hours)	6.1	6.1	5.6	5.7	8.4	7.7
Rainfall (mm)	0.0	4.0	0.0	72.9	16.7	1.3

are average of three readings from each of the experimental unit. Spike length, number of spikelets per spike and number of grains per spike were computed from each plot by taking the twenty samples and average was taken. Each observation of 1000 grain weight, given as replication is average of three measurements. To record biological yield, a unit area in each plot was reaped manually, sun dried until constant weight, packed and weighed. After recording biological yield, the all the packs were threshed manually and grain yield was recorded. Harvest index (HI) was calculated according to the formula

$$\text{Harvest Index (HI)} = \frac{\text{Grain yield}}{\text{Biological yield}} \times 100$$

Statistical analysis: Collected data were subjected to the analyses of variance to depict the difference among treatments followed by Least Significant Difference (LSD) test at 5% probability to compare the differences among means of treatments (Steel *et al.*, 1997). Moreover, Microsoft Excel 2003 was used to make graphs and Fig. s along with standard error.

RESULTS

Seed priming and size showed a significant ($P \leq 0.05$) while terminal drought stress showed non-significant ($P \geq 0.05$) effect on early stand establishment (Fig. 1). Seeds treated with calcium chloride (CaCl_2) significantly ($P \leq 0.05$) lowered the time to first emergence and mean emergence time (MET) for both bold and small sized seed lots compared with dry seeds (Fig. 1a and b). Moreover, bold seeds compared with small sized seeds and osmoprimed seeds compared with dry seeds noted the minimum time to start emergence (E_{50}) both under well-watered and terminal drought conditions (Fig. 1c). However, osmoprimed seeds compared with dry (control) seeds observed higher emergence count for both bold and small seed lots under normal irrigated and water stressed conditions (Fig. 1d).

Terminal drought stress lowered the LAI, at 85 and 105 DAS, compared with well irrigated conditions. Nonetheless seeds primed with CaCl_2 significantly ($P \leq 0.05$) improved the LAI during whole the growth duration both under well-watered and terminal drought conditions. However, seed size had non-significant effect on LAI (Fig. 2a). Likewise, terminal drought stress curtailed the LAD while osmopriming significantly ($P \leq 0.05$) improved the LAD both under irrigated and water stressed environment compared with dry seeds of both sized lots (Fig. 2b). Similarly, drought stress compared with well-watered conditions lowered the CGR at 65-85 and 85-105 DAS while osmopriming and bold sized seeds improved the CGR particularly under stress conditions (Fig. 3a). Moreover, terminal drought impaired the NAR of wheat while osmopriming and bold sized seeds improved the NAR particularly under stress conditions (Fig. 3b).

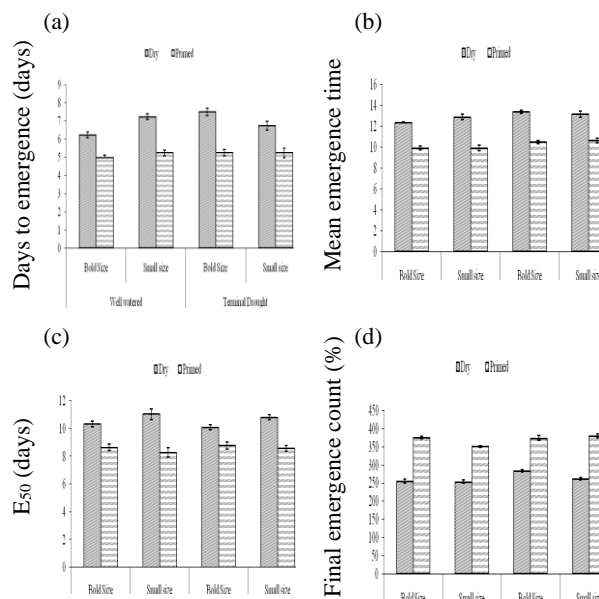


Figure 1. Effect of seed size and seed priming on days to start emergence (a), MET (b), E_{50} (c) and final emergence count (d) of wheat sown under well-watered and terminal drought stress.

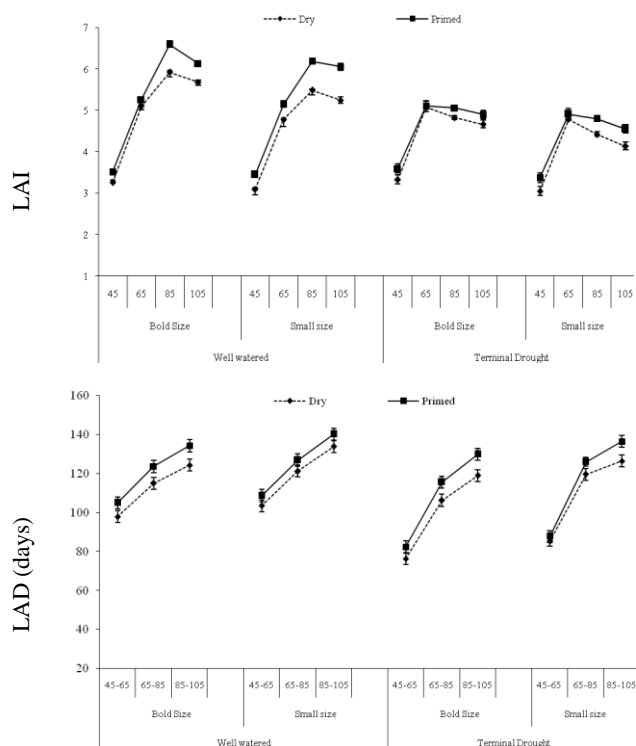


Figure 2. Effect of seed size and seed priming on leaf area (LAI) index and leaf area duration (LAD) of wheat sown under well-watered and terminal drought stress.

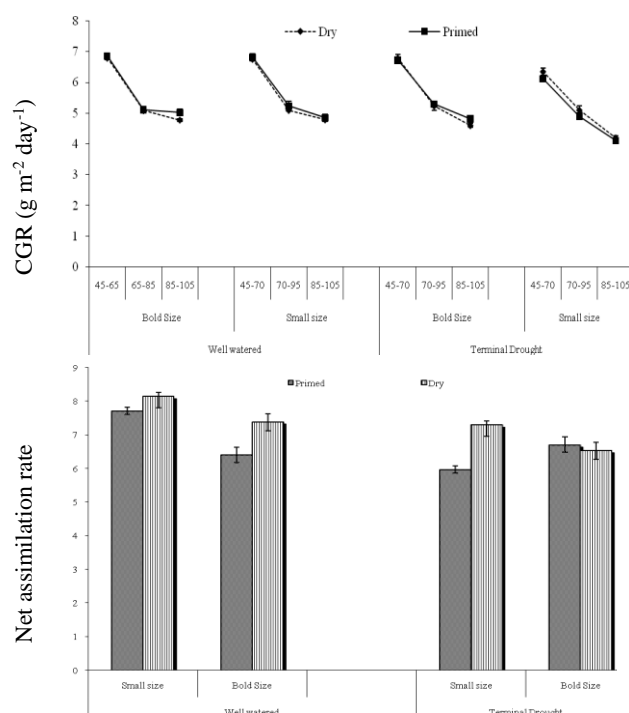


Figure 3. Effect of seed size and seed priming on crop growth rate (CGR) and net assimilation rate (NAR) of wheat sown under well-watered and terminal drought stress.

Interaction among seed size, priming techniques and drought had a significant ($P \leq 0.05$) effect on yield related traits (Table 2). Wheat sown with osmoprimed bold seeds under well-watered conditions had the maximum while wheat sown with small dry seeds under terminal drought observed the minimum plant stature (Table 2). Likewise, wheat sown with bold seeds either primed or not observed long spikes under well-watered environment while wheat sown with dry small seeds subjected to terminal drought stress observed small spikes (Table 2). Under well-watered conditions, wheat sown by primed and dry seeds of both sizes observed higher population of productive tillers while crop sown by small sized seeds, either primed or not, recorded lesser population of productive tillers subjected to terminal drought (Table 2). Bold sized seeds either primed or not while small sized osmoprimed seeds observed more number of spikelets and grains per spike under well-watered conditions. However small sized dry seeds observed the minimum number of spikelets and grains per spike under terminal drought stress (Table 2). Nonetheless, osmoprimed bold sized seed noted the highest 1000-grainweight while the crop subjected to terminal drought had small weight grains either sown by primed or dry seeds of both sized categories (Table 2).

Interaction between seed size, priming techniques and drought had a significant effect ($P \leq 0.05$) on biological yield, straw, grain and harvest index of wheat (Table 2). Bold sized seeds either primed or not, and osmoprimed small sized seeds

Table 2. Effect of seed size and seed priming on plant growth, biological yield, grain and straw yield and harvest index of wheat sown under well -watered and terminal drought stress.

Seed priming techniques	Plant height (cm)				Spike length (cm)			
	Water stress				Water stress			
	Well -watered		Terminal Drought		Well -watered		Terminal Drought	
	Bold	Small	Bold	Small	Bold	Small	Bold	Small
Dry Seed	97.47 ab	97.71 ab	94.68 ab	91.46 b	11.53 a	10.79 bc	10.32 d	9.43 e
Primed Seed	99.06 a	96.91 ab	94.07 ab	94.32 ab	11.66 a	10.96 b	10.52 cd	10.16 d
LSD at 5 % probability level	6.44				0.37			
	Spikelets per spike				Number of grains per spike			
Dry Seed	18.01 a	17.09 b	16.72 bc	15.25 d	43.25 ab	40.50 cd	40.60 bcd	38.95 d
Primed Seed	18.23 a	18.29 a	16.45 c	16.95 bc	44.17 a	44.55 a	42.05 abc	40.00 cd
LSD at 5 %	0.53				2.65			
	Number of productive tillers (m^{-2})				1000-grain weight (g)			
Dry Seed	360.36 a	344.48 abc	331.68 bcd	302.38 e	39.62 b	38.75 b	37.00 c	36.50 c
Primed Seed	355.17 ab	348.35 abc	326.25 cde	315.58 de	40.75 a	39.50 b	37.50 c	37.37 c
LSD at 5 %	27.79				1.09			
	Biological yield ($kg\ ha^{-1}$)				Grain yield ($kg\ ha^{-1}$)			
Dry Seed	12487 a	11440 bc	10705 cd	9405 e	4269 ab	3807 c	3752 c	2476 e
Primed Seed	12463 a	12300 ab	11565 abc	10100 de	4510.3 a	4236 ab	4202 b	2876 d
LSD at 5 %	978.24				278.85			
	Straw yield ($kg\ ha^{-1}$)				Harvest index (%)			
Dry Seed	8218 ab	7633 bc	6903 de	6649 e	34.19 ab	33.27 b	35.25 ab	27.18 c
Primed Seed	8509 a	8226 ab	7363 cd	7038 cde	34.66 ab	34.06 ab	36.37 a	28.99 c
LSD at 5 %	624.13				3.05			

observed higher wheat grain yield under well-watered conditions while small sized dry seeds recorded lesser grain yield of wheat subjected to terminal drought stress (Table 2). Bold sized seeds either primed or not, and osmoprimed small sized seeds observed higher straw and biological yields of wheat under well-watered conditions while small sized seeds either primed or not recorded the lesser straw and biological yield of wheat subjected to terminal drought stress (Table 2). Crop sown by using bold sized seeds either primed or not observed higher harvest index under optimal and stressful environment but it was at par with the crop sown by small sized primed seeds under well-watered conditions. However crop sown by small sized seeds, either primed or not, subjected to terminal drought stress had the minimum harvest index (Table 2). Wheat put under terminal drought stress observed substantial decrease in grain filling rate; however both sized seeds, bold size in particular, osmoprimed with CaCl_2 counteract the damaging effects of drought on grain filling rate (GFR) and observed higher grain filling rate both under optimal and stressful environment (Fig. 4).

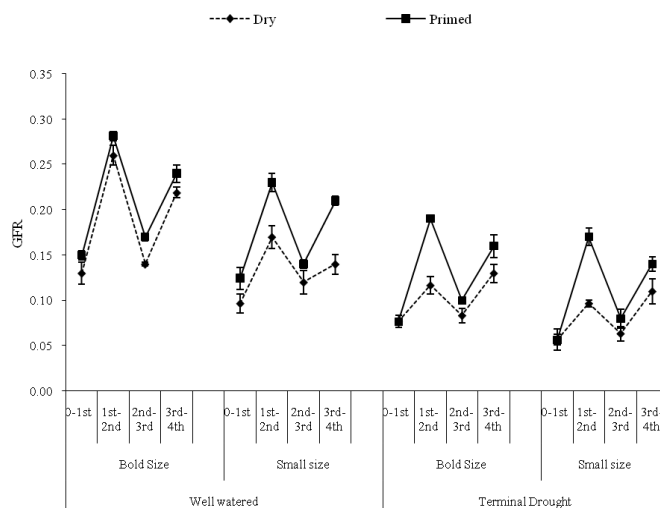


Figure 4. Effect of seed size and seed priming on grain filling rate of wheat sown under well-watered and terminal drought stress.

DISCUSSION

Terminal drought stress impaired the grain yield of the crop due to significant ($P \leq 0.05$) decrease in yield related parameters of wheat (Table 2). Nonetheless osmopriming of seeds with CaCl_2 , bold seeds in particular, improved the wheat productivity owing to significant ($P \leq 0.05$) increase in allometric and yield related parameters under well-watered and terminal drought conditions (Figs. 2-3; Table 2).

Grain yield of wheat is the outcome of various morphological traits like productive tillers/unit area, spikelets per spike, grains per spike and grain size. In this study, wheat grown under terminal drought observed a heavy yield penalty due to

substantial reduction in the performance of morphological traits related to yield (Table 2). Bread wheat productivity is seemed highly sensitive to terminal drought stress due to notable cut in grains count and size (Sivamani *et al.*, 2000; Milad *et al.*, 2011; Farooq *et al.*, 2014, 2015). In this study, decreased number of grains per spike under terminal drought might resulted in the response of reduced number of spikelets per spike; as number of spikelets per spike determines the grains count in wheat (Farooq *et al.*, 2014). However, pollen abortion is another reason of reduced number of grains per spike facing terminal drought stress (Farooq *et al.*, 2014). Moreover, decrease in 1000-grain weight noted in this study was due to substantial cut in grain filling rate under terminal drought (Fig. 4) because grain filling rate and duration are the two important motives to determine grain size in wheat (Farooq *et al.*, 2014). Crop subjected to terminal drought observed small LAI at lateral phase of growth which might be due to reduced water supply leading to decrease in leaf turgor (Hussain *et al.*, 2009; Farooq *et al.*, 2015). As plant leaves act as units of food factory of plants, therefore decreased LAI resulted in small interception of sunlight which ultimately resulted in lower accrual of photo-assimilates. All this resulted in decreased CGR and NAR which ultimately resulted in reduced grain filling rate tied with small grain size and yield penalty under terminal drought stress (Farooq *et al.*, 2015).

Osmopriming resulted in early and more uniform stand establishment compared with dry seeds as germination, emergence and growth of primed seeds are quicker than non-primed ones (Shad *et al.*, 2001). Homogeneity and quicker emergence results by seed priming due to elevated enzyme activation and early start of seed metabolism (Khan *et al.*, 2008; Khalil *et al.*, 2010). Early and uniform emergence of primed seeds results in efficient utilization of resources and hence performed better during entire growing period under optimal and stressful environment. In addition, osmopriming enhanced LAI leading to higher CGR under well irrigated and terminal drought conditions (Fig. 2 and 3) due to more sunlight interception. Therefore 1000-grain weight was improved due to substantial rise in CGR, NAR and grain filling rate as developing grains found more assimilates compared with dry seeds. Improvements in wheat performance by osmopriming, particularly with CaCl_2 , have been observed in earlier studies under well-watered and drought CaCl_2 has earlier been reported (Farooq *et al.*, 2008, 2015).

Bold sized seeds used in this study improved the emergence count and also resulted in uniform and early seedling emergence (Fig. 1). Seed weight plays an important role during emergence and further seedling growth. There is a constructive and important correlation among seed weight and seed germination percent (Khan, 2003). This positive relation among seed weight and emergence may be because of more food reserves in seed. This idea is supported by

Westoby *et al.* (1996) who reported that there are more mineral nutrients and organic reserves in bold seeds compared to small seeds. Moles and Westoby (2004) noted that bold seeds results in plant having more survival rate as compared to smaller seeded ones. Hence, wheat crop emerged from bold seeds produced early seedlings with more leaf area dry matter accumulation (i.e. LAI and CGR; Fig. s 2 and 3), bold grains, and higher grain and biological yield than the crop emerged from small seeds (Tables 2 and 3; Royo *et al.*, 2006; Dohuki *et al.*, 2011). From 4.2 to 16% increase in bread wheat productivity by growing bold seeds compared to shrunked seeds is well reported (Chastain *et al.*, 1995; Royo *et al.*, 2006). Spike traits like number of spikes per unite area and spike length enhanced by bold sized seeds is supported by the findings of Royo *et al.* (2006) findings who reported more and production of plants produced from bold seeds.

Conclusion: In conclusion, terminal drought stress substantially decreased wheat productivity in response to reduction in allometric and morphological traits; nonetheless, osmoprimed seeds with CaCl₂ over dry seeds and large sized seeds over small sized seeds maintained their dominance due to early and uniform stand establishment, improving crop allometry and productivity due to significant expansion in all yield related parameters under well-watered and drought stress conditions. In conclusion, osmoprimed bold sized seeds can play a dynamic role in improving wheat production by early and uniform stand establishment, improving crop allometry and productivity.

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