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Original Research

Effects of Priming on Seed Germination, Physico-Chemistry and Yield of Late Sown Wheat Crop (*Triticum aestivum* L.)

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Abstract

Wheat productivity is drastically affected by late sowing, as it faces high temperatures during grain filling which decreases the crop yield. In this experiment, several seed priming agents were used to improve performance of two wheat varieties under normal and late sowing regimes. Hydro-priming, Osmopriming with 2% calcium chloride and hormonal priming with salicylic-acid (50 mg/L) used as seed priming treatments. All treatments resulted in better seedling establishment, physiological attributes, growth and yield as Salicylic-acid proved superior in synchronized seedling stand establishment by reducing time required for 50% emergence and mean emergence under both sowings. Overall, growth and yielding traits were improved in both varieties subjected to seed priming with salicylic-acid and $CaCl_2$ under normal and late sowings. Salicylic-acid and $CaCl_2$ priming showed highest proline, sugars, phenolics, chlorophyll and relative water contents in Sarsabz and Khirman, while hydro-priming gave maximum glycine-betaine and membrane thermo-stability. Sarsabz showed more tolerance against high temperature. Seed priming improved high temperature tolerance under late sowing; however, $CaCl_2$ and salicylic-acid priming remained more effective in mitigate drastic effects of high temperature by maintaining better growth and yield as well as improved physiological attributes in both varieties. These enhancers can give more production under adverse climatic conditions.

Keywords: climate change, hydro-priming, late sown crop, osmo-priming, water stress

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Introduction

Wheat is the 3rd most growing, cereal crop of the world after maize and rice, in South Asian countries it is considered as main source of nutrition for ever increasing population as staple food. Cultivation of the crops through conventional techniques, proved to be not successful in fulfilling the demands of the food globally. Along with these conventional techniques, which are mostly practiced in subcontinent countries, drastic change in climate over the years in this region specifically contributed to change of cropping patterns which is now resulting in loss of crop yield. As the plants are facing tremendous increase in temperature of globe, which is resulting into the melting of glaciers, floods, change in weather conditions and resultantly affecting the phenology (Life Cycle) of the plant [1]. Among these factors, unprecedented rise in temperature globally, is affecting the yield of the crop. It is evaluated that with every 1ºC of temperature will not only contribute to global warming and drought, but it will be the main cause of wheat reduction by 6% annually [2]. This could be possible due to change in concentration of water and temperature, both components are essential for growth and development of plants, their influence can be seen in regulation of gene expression both by down regulating and up regulating, the several enzymes and proteins of the plant's metabolic activities [3]. While the shortage of water supplies to the crop plants found to result in delay of germination of sunflower and pepper [4]. Moreover, it was also reported that pollen sterility, reduction in flower and seed size are due to the shortage of water supply to the crop plants [5].

Wheat cultivation in Pakistan is mainly carried out in two sowing patterns, on is practiced in late October and in early November (Early Sowing) and second one in mid-December or in last week of December (Late Sowing). This late sowing of wheat previously reported to have later emergence of seedling, reduced tiller density, less numbers of grains and reduced weights of the seeds which is resulting into the decrease of the crop yield [6]. This is mainly due to fluctuations in temperature and time of growth period which effects the productivity of the crop [7]. Previous studies narrated, negative impact of the high temperature on emergence of crops [8]. Late sowing of the wheat results in exposure of high temperature at the time of grain filling. That's why life cycle of late sown crop become shortened as the process of accumulation of starch in seed become completed both in optimal and late planted crop plants. Plants response against high temperature at its developmental stages alter morphological development, assimilation modulation, antioxidant activities, protein synthesis and disruption in functionality of cellular membranes [9].

In Pakistan late sowing of the wheat crop in late December in most parts of the country is mainly due to the late harvesting of the preceding crops and lack of pre sowing irrigation water [10]. In various research works it has been shown that hydropriming of field crops resulting into maximum germination of seedling growth, seedling dry weight and germination time of seedling [11].

Different seed priming techniques used various exogenous applications which may be helpful in improving the stand establishment, physiological, yield and quality parameters of plants under late sown conditions [12]. This technique is quite simple and cost effective in which seeds are hydrated partially until the initiation of metabolic activities required for the germination of the seed, with the emergence of radical and plumule. In this way metabolism of endospermic starch is reduced resulting into uniform emergence of seedlings. This process results in high production of secondary metabolites, antioxidants, activation of enzymes such as Lipase and Amylase [13]. Osmopriming with $CaCl_2$ improves seedling stand establishment and yield performance through physiological enhancement under late sown conditions [14]. Salicylic acid (SA) is one of the major plant hormones which promote growth, development, and yield by enhancing cell division in apical meristem of many crops under extreme environment. Various research works in different parts of the world were carried out to address the issue of the tolerance of the wheat and other crops against drought, which are briefly described in the following lines. The effect of the priming techniques was studied on late sown wheat in research farms of the district Sheikhupura (Pakistan). It was concluded in experiment of three years data of crop that late sown wheat seeds should be treated with priming materials, so their production of the number of seeds and weight of the seeds was found improved in this experiment [15]. Impact of various applications of the priming was checked on two varieties of wheat i.e., Adana-99 and Pandas for their agromorphological attributes and during the experiment it was observed that among various techniques PEG (Polyethylene Glycol), KCl (Potassium Chloride) and Hydropriming techniques gave tremendous results in late sown wheat crop [16]. Moreover, effect of the priming durations and techniques was also studied on behaviour of melon germination under saline temperature stress. The priming technique used were $CaCl_2$, KNO_3, K_3PO_4, NaCl and Polyethylene glycol. The best germination was observed with $CaCl_2$ after 3 days [17]. The germination rate and production of the *Abelmoschus esculentus* L. under two priming techniques (PEG and Mannitol) was carried out in which the significant increase in the yield of the primed seeds were observed as compared to the unprimed seeds [18]. These seed priming applications are even better in promoting the conservation techniques of various tropical trees e.g., seedling performance of four semideciduous trees were analysed in Mexico. Results of hydropriming and natural priming on seeds of *Swietenia macrophylla, Enterolobium cyclocarpum,* *Cedrella odorata* and *Albizia saman* were analysed through their water, mass and lipid content and it was concluded that hydropriming of *E. cyclocarpum* can give best germination results and natural priming can yield better germination results in rest of three species [19]. Effect of the seed priming on early growth and germination of the seeds of *Helianthus annus* L. was studied in which priming with hydrosulphide was carried out in drought conditions. it was observed that relation between the drought stress and germination of seeds, where the drought conditions were inhibited, early germination of seeds were recorded [20]. Various studies have been carried out in various parts of the world regarding impact of climate change on tree species, endangered ornamental species and tree species which signifies the importance of such studies which are related with the development of cheaper methodologies for the germination of the seeds in rapidly changing environmental conditions few examples of are reported in the following lines. Endemic, vulnerable plant species like *Lailium martagone* L. were treated with growth hormones i.e., Gibberlic Acid (GA) Nophtyl acetic acid (NAA); Idol Acetic Acid (IAA) and Indol Butyric Acid (IBA) and their effect was observed by six morphological attributes of the *L. martagon* species by applying three different doses at basal, medium and apical parts. Among these doses 1000 ppm doses of IBA was found to have more efficacy on morphological characteristics. Along with this all these parts showed great potential for meristematic activity [21]. While importance of economically important wild species of *Lilium artrinense* cannot be ruled out as its population was reportedly decreasing in nature. So, they tried to develop cheap methods of seed germination. Total 39 applications of four hormones i.e., IAA, IBA, NAA and GA3 was given to meristematic parts of the species which induced two times greater growth than the normal [22]. Seed germination of nine ornamental plant species i.e., *Ailanthus altissima*, *Cupressus awnizonica*, *C. sempervirens*, *Kolelreuteria paniculata*, *Pirus brutia*, *P.nigra*. *Pyracantha coccinea*, *Sophora japonica* and *Thuja oricentalis* was carried out in water stress conditions. It was observed that germination percentage decrease from -2 bar water resistance in each species. The most water stress resistant species were *P. nigra*, *C. sempervirens* and *P. brutia* [23]. Different variations among morphological traits of *Euonymus japonius* cultivars were recorded which signifies that Cultivar variegate had the highest percentage rooting and green Rocket had the lowest level among all cultivars Green Rocket performance was better than all others under extreme changing climatic conditions [24]. The sufferings of human beings is linked with rapid changes in climate, mainly due to extensive industrialization. The role of forests in reducing the ever-increasing pressure of climate change has enhanced over the years. This can be achieved by adopting sustainable approaches in increasing the structural quality of the forest. In the present study forest species with best cone yield and growth rate was determined. This was carried out by taking diameter and height of *Pinus sylvestris* L. cones of Erzurun region [25]. Climate change impacts related with UV-B radiations are getting into limelight as it reaches the earth's surface quicker due to deterioration in ozone layer around the world. The study was conducted to check the effects of UV-B on germination of black pine seed. It was concluded that variable induction of UV-B radiation cause reduction in all characters even at minimum exposure time [26]. The role of variable climates in seed germination cannot be ruled out specifically in case of extinct species like *Juniperus drupacea* (Labill.) Ant. et Kotschy. Results of the study indicated that seeds from higher altitudes showed higher rate of germination in all pre-treatments. So, it was concluded that seeds for germination should be considered from higher altitudes due to their higher germination rates [27]. Moreover, it was observed that micromorphological attributes of five different ornament woody species were affected in variable climatic conditions. In leaf characteristics, length, width, and density of stomata was considered along with their pore width and length. Variable results in different climatic conditions were observed in terms of their characteristics. Based on these results, it was concluded that different species react differently according to their climate type [28].

Keeping in view the water scarcity, vulnerable climatic conditions and natural catastrophes, Pakistan is facing tremendousl and specific climate change impact on cropping pattern in arid and semiarid regions, this study was designed with the objectives to evaluate various seed priming techniques for improving seed germination rate and higher grain yields as well as mitigating the adversities of high temperature imposed in late sowing.

Materials and Methods

Experimental Design

Studies on effect of priming in two wheat varieties (Khirman & Sarsabz) were carried out in Nuclear Institute of Agriculture (NIA), Sindh, Pakistan. For this purpose, seeds were obtained from Plant Breeding & Genetics Division of NIA. Pot experiment was conducted under net house, and these pots were arranged following the completely randomized design (CRD) with three replications. Late sowing was used as high temperature treatment as crop faced more than 35ºC temperature during grain filling. Each pot was filled with 10 kg clay loam soil, with 0.49 dS/ m EC and pH 7.2. Seeds were sown on 16th November (normal sowing) while late sowing was done on 16th December. Soil temperature and weather data were also recorded (Fig. 1).

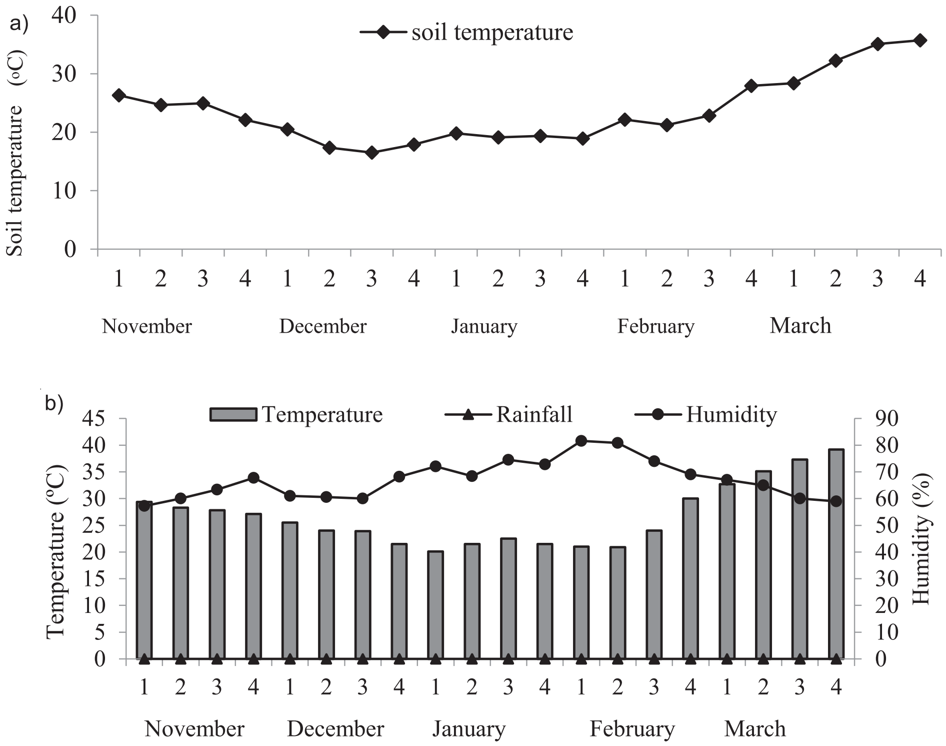

Fig. 1. Weekly soil temperature a), atmospheric temperature, rainfall and humidity data b).

Priming Treatments of Seeds

Priming treatments of the seeds (Experimental) consists of three priming techniques i.e., Hydropriming (with distilled water), Osmo-priming (with 2% $CaCl_2$), and hormonal priming (with 50 mg/L salicylic acid), whereas non primed, dry seeds were selected as control. Seeds were primed with respective osmotica by keeping 1:5 (w/v) seed to solution ratio for 12 h (hours) at room temperature. These soaked seeds were washed thrice with distilled water and dried up under shade till the initial seed moisture was restored [29].

Seed Germination (Emergence)

Emergence of the germinated seeds was counted with various parameters like, 50% Emergence (E_{50}), Mean Emergence Time (MET), Emergence Index (EI) and Final Emergence Percentage (FEP) accordingly on daily basis [30]. The formulae of these above mentioned parameters are given below.

$$\text{50\% Emergence } (E_{50}) = \frac{t_i + [\frac{N}{2} - n_i]}{n_j - n_i} \times (t_j - t_i)$$

Whereas N = final emergence count
n_i = cumulative number of emerged at adjacent days t_i and t_j when $n_i<(N+1)/2<n_j$

$$\text{Mean Emergence Time } (MET) = \Sigma Dn / \sum n$$

Where, n = emerged seeds number,
D = number of days calculated from the start of sowing

$$\text{Emergence Index } (EI) = \left(\frac{No.of seeds germinated}{Days of first\ count} + \cdots \ldots \ldots + \frac{No.of seeds germinated}{Days of final count}\right) \times 100$$

$$\text{\% Final Emergence Percentage } (FEP) = \frac{Final\ number\ of\ seedlings\ emerged}{Total\ number\ of\ planted\ seeds} \times 100$$

Growth Parameters

Several growth parameters like Tillers per plant, Productive tillers, Leaf area (cm^2) and Plant height was recorded.

Yield Traits

Yield of the crop was estimated through various parameters like, Number of grains per plant, 1000-grain weight, Grain yield, Harvesting Index and Biological Index.

Physiological Analysis

Relative water contents (RWC), cell membrane thermo-stability were estimated at various developmental stages of crop (60, 75 and 90 DAS) by adopting the methodology of [31].

% Relative Water Content (%RWC)

RWC was determined on the basis of fresh, turgid and dry weights of flag leaves through following formula:

$$RWC(\%) = \frac{W_f - W_d}{W_t - W_d} \times 100$$

Where;
W_f = Fresh leaf weight
W_t = Turgid leaf weight after dipping in water for 24 h
W_d = Dry leaf weight after oven drying at 70ºC for 24 h

Cell Membrane Thermo-Stability

Leaf samples were soaked for 12 h in 10 mL distilled water and EC_1 (Electrical Conductivity) was measured. Then at 60ºC the leaf samples were heated in water bath and cooled up to 25ºC and again EC_2 was measured. Ratio of both these EC1 and EC2 is expressed as Cell membrane thermos-stability.

Chemical Analysis

It was carried out through Chlorophyll, Proline, Glycine-betaine (GB), Total Soluble Sugar (TSS) and Phenolic compounds determination by the standard procedures.

Chlorophyll Contents

Chlorophyll contents were analyzed by homogenizing fresh leaves in 80% acetone solution for 24 h in dark and optical densities (OD) of filtered solutions were read at 663 nm and 645 nm by using Hitachi UV-4000 spectrophotometer [32].

Estimation of Proline

Fresh leaves (0.5 g) were taken to homogenize with 10 mL of 3% sulpho-salicylic acid solution and then filtered. Out of these plant extracts, 2 mL of each plant sample was heated with ninhydrin and glacial acetic acid solution at 98 °C in water bath for an hour and cooled instantly. After that, 4 mL toluene was poured in above mixture and shaked vigorously for 20-30 sec. As a result, two layers were formed. Upper pink colored layer was separated for measuring OD at 520 nm against blank [33].

Estimation of Glycine-Betaine (GB)

It was measured by taking the extracts of fresh leaf samples (0.5 g) through 0.5% toluene solution (10 mL) by shaking it for 3 h. Then 1 mL of Plant extract was reacted with 2 N HCl (0.5 mL) and 0.2 mL of KI_3 in shaking ice-bath for about 1.5 h. After that, 2 mL of distilled water, along with 10 mL of 1-2, dichloro-ethane was poured in above mixture and vortexed for few seconds. OD of lower reddish-brown layer was read at 365 nm through spectrophotometer by the standard procedure [34].

Total Soluble Sugars (TSS)

Fresh plant material i.e., 0.5 g was immersed in 80% ethanol (5 mL) and shaked mechanically for 3 h. Then 0.1 mL plant extract was reacted with anthrone (3 mL) solution by heating for 10 mins. in water bath at 95ºC, and then placed in an ice bath for instant cooling. OD of transparent green samples was taken at 630 nm [35].

Phenolic Compounds

Dried leaves (0.5 g) were extracted in 80% acetone (5 mL), then 40 mL of sample extract was taken, and 3 mL distilled water was added with 200 µL Folin Ciocalteu reagent in a test tube. After this 2 M Na_2CO_3 (600 µL) solution was poured in above solution after 5-8 min. Test tubes were incubated at 40ºC for 30 min. and OD was measured at 765 nm by the standard methodology [36].

Statistical Analysis

All the data obtained in triplicates from the above mentioned parameters and thus analyzed through Duncan's multiple range test (DMRT) at $p \leq 0.05$ [37] by using Statistix 8.1 software.

Results and Discussions

Soil temperature and metrological data had shown fluctuations throughout the wheat growing season. There was a sharp decline in internal temperature of soil during the months of December and January which had reduced the seed emergence of late sown treatment. However, there was zero rainfall recorded during the whole wheat season 2015-2016 in Tandojam city. Humidity was recorded between 57% (lowest) to 81% (highest). Meanwhile, air temperature showed abrupt increment from 24ºC to 30ºC in the last week of February and 40ºC temperature was recorded at the time of crop harvesting (Fig. 1).

Seed germination and seedling stand establishment are critical processes in crop productivity. Seed treatments significantly improved the performance of both varieties under optimal and late sowings. Time required for 50% emergence of seeds was significantly reduced for both varieties under SA and $CaCl_2$ treatments. This might be due to enhanced seed metabolic activities which improves germination. Similarly, MET was also effectively reduced by SA-priming in Sarsabz and Khirman at both sowing dates. Higher EI were recorded by SA seed treatments. Early seed emergence indicated by lower MET and E_{50} values might be due to enormous biochemical

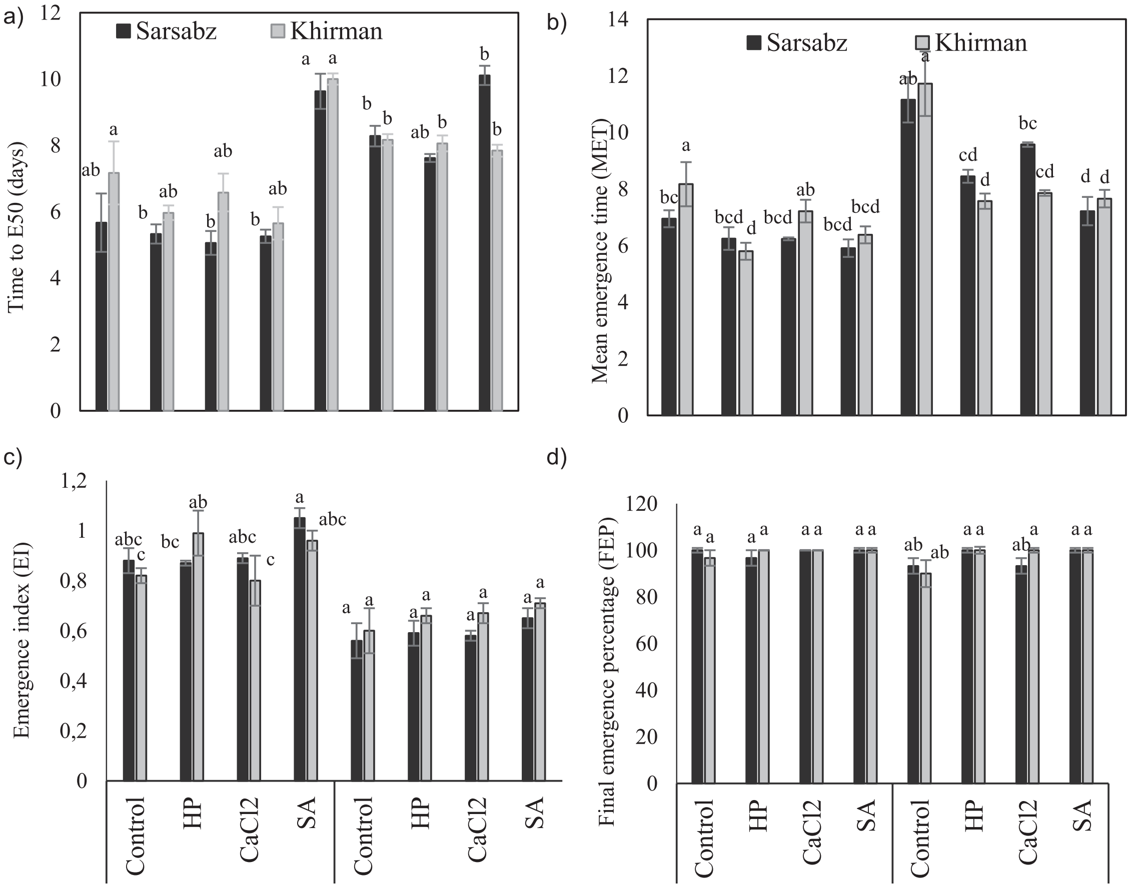

Fig. 2. Effect of various seed priming treatments on time required for 50% emergence a), mean emergence time b), emergence index c) and FEP d) of both Sarsabz and Khirman wheat varieties under normal and late sowings conditions.

modification such as hydrolysis and enzyme activation, occurring under the influence of seed priming. Despite of significant improvement in seedling quality traits compared to control, no significant differences were observed among hydro-priming, $CaCl_2$ and SA priming in case of FEP (Fig. 2). Moreover, poor germination in non-primed seeds might be the result of slower metabolic activities particularly under late sowing. Mostly the crops with slow seedling growth or delay crop emergence enhances the risk of attack of pests it ultimately reduces the yield of seed.

Previous research proved higher germination rate of seedling applying hydro-priming germination procedures including time of germination and germination index [38]. Furthermore, hydro-priming can stimulate germination, specifically in unfavourable conditions, better germination rate in many crop species [39].

Different varieties of wheat contain diverse growth habit and yield formation with respect to sowing time and rampant climatic conditions [7]. In this experiment, plant growth was principally affected by temperature. More tillers, productive tillers, leaf area and plant heights were observed in normal sowing compared to late sowing. High temperature has reduced plant height in late sown crop due to accelerated heading. Among treatments, SA and $CaCl_2$ priming further enhanced growth traits under normal sowing however, in case of late sowing, results of all these chemicals were statistically significant in improving the crop growth (Table 1). Increased LA by seed priming might have enhanced yield due to more interception of light by canopy. These findings were further supported by previous study of Assefa and Hunje [44]. which highlighted the increased growth of soybean through $CaCl_2$ osmoprimed seeds under high temperature.

In tropical and dry climatic conditions, timing of phenological events is directly associated with various environmental factors such as soil and air temperatures. Flowering and grain filling are the critical stages for wheat crop yield especially under late sowings because it has to face thermal stress at reproductive stage. Yield attributes such as grains per plant, 1000-grains weight, harvesting and biological indices were significantly higher in SA priming followed by $CaCl_2$ priming. Nevertheless, more reduction in grain yield occurred in late sown crop with slight enhancement by priming treatments. During later developmental stages in late planting, high temperature is linked with early leaf senescence which reduces the process of photosynthesis; hence less photosynthates will be translocated towards the developing grains and producing shrunk grains.

Table 1. Effect of various seed priming treatments on growth attributes of both wheat varieties (Sarsabz and Khirman) under Normal and Late sowing.

Treatment		Tillers per plant		Productive tillers		Leaf area (cm^2)		Plant height (cm)	
		Sarsabz	Khirman	Sarsabz	Khirman	Sarsabz	Khirman	Sarsabz	Khirman
Normal Sowing	Control	3.11±0.29cd	2.78±0.11d	2.67±0.19cd	2.78±0.11d	20.44±1.19b	26.51±1.22a	71.3±0.21a	62.33±0.43a
	Hydro-P	3.78±0.40bc	3.56±0.29c	3.22±0.22de	3.44±0.22cd	25.91±1.25ab	27.5±0.72a	76.67±1.13a	74.3±1.12b
	$CaCl_2$-P	3.56±0.11c	4.33±0.33ab	3.56±0.11bcd	3.89±0.11abc	26.69±2.63a	30.45±3.12a	78.672.45±ab	80.0±2.18c
	SA-P	4.33±0.19ab	4.67±0.19a	4.11±0.40a	4.00±0.19ab	24.92±2.29b	25.40±2.16ab	80.0±1.75 a	81.0±1.98bc
Late Sowing	Control	1.67± 0.82f	1.22±0.55b	1.00±0.02b	1.33±0.22b	6.70±0.78d	9.45±0.66c	54.0±2.11b	48.67±3.11d
	Hydro-P	1.78±0.93b	1.89±1.07b	1.67±0.33ab	1.89±0.33ab	7.45±0.79d	11.55±0.70b	57.0±1.43a	56.0±1.94bc
	$CaCl_2$-P	1.78±0.45b	1.33±0.43a	1.33±0.11ab	1.00±0.01b	12.9±0.71ab	13.30±1.11ab	59.0±0.98a	62.67±0.32a
	SA-P	2.89±1.30b	1.67±0.54b	2.00±0.41ab	1.67±0.34ab	13.36±2.03a	12.00±0.42ab	67.3±.71a	66.67def
LSD ($P \leq 0.05$)		0.8171		0.6611		3.8661		0.7761	

Abbreviations: Hydro-P = Hydropriming; $CaCl_2$-P = Calcium chloride priming; SA-P = Salicylic acid priming

Mean value followed by various alphabets represents significant differences (LSD test, $p<0.05$) between various treatments under both sowing conditions (Duncan test, $p<0.05$).

Reduced grain weight is also found to be associated with endospermic and aleurone layer alterations under high temperature [40, 41]. Maximum grain yield was recorded in Sarsabz when primed with SA under normal as well as late sowing, respectively. Among wheat varieties, Sarsabz was found superior than Khirman in terms of grain yield. SA priming was more effective followed by $CaCl_2$ in late sowing while, under normal sowing, $CaCl_2$ proved better than SA (Table 2). Many researchers had used various seed priming treatments for early seedling establishment, improved growth in terms of more productive tillers, 1000-grains weight and grains per spike which ultimately resulted in higher grain yields under abiotic stresses [42]. Wheat production is increased by enhanced translocation rate of photosynthates towards grain under the influence of hormonal priming. Augmentation in biological yields and harvesting indices through seed priming was due to enhanced dry matter partitioning towards spikelets. The successful effects of Seed priming were found in maize and wheat [43].

Water is the prerequisite for crops; however, high temperature has direct effects on plant water relations. Evapo-transpirational losses were enhanced under high temperature causing dehydration damages. Seed priming proved highly effective in increasing RWC at various growth stages under both sowings. Upmost RWC were observed under all treatments in Sarsabz while Khirman's seed treatment with water at 75-DAS constituted highest RWC under normal conditions. In delayed sowing, maximum RWC were determined at 60-DAS in both varieties (Fig. 3). Higher flag leaf RWC were maintained at initial crop development stages while reduced gradually towards crop maturation due to dry matter accumulation. Seed treatments were proved effective in sustaining high RWC at all crop growth stages compared to control. Similar higher RWC were observed in seed primed with $CaCl_2$ in soybean [44].

Cell membrane permeability was also modulated by seed priming treatments. Hydro-priming proved best in maintaining integrity of cell membrane of Khirman under normal sowing. However, in case of late sowing, these seed treatments proved more effective in reducing membrane permeability at 60-DAS as compare to 75 and 90-DAS. $CaCl_2$ priming treatment only maintained the membrane integrity at 90-DAS in both varieties (Fig. 3). High temperature causes disintegration of thylakoid membranes which decreases efficacy of membrane associated enzymes and electron carriers, hence reduces photosynthesis. More stable membranes exhibited slower electrolyte leakage even under higher temperatures. Reduction in membrane permeability in plants primed with SA was due to better membrane structures maintained by Ca^{+2} accumulation and slow hydration [45].

Photosynthesis is quite sensitive to temperature rises, even short exposure of high temperature can cause inhibition of oxygen evolving complex and

Table 2. Effect of seed priming treatments on yielding attributes of wheat varieties (Sarsabz and Khirman) under Normal and Late sowing.

Treatments		Number of grains per plant		100 grain weight (g)		Grain yield (t ha^{-1})		Harvesting Index (%)		Biological Index	
		Sarsabz	Khirman	Sarsabz	Khirman	Sarsabz	Khirman	Sarsabz	Khirman	Sarsabz	Khirman
Normal Sowing	Control	85.33±2.91b	91.56±1.66b	3.72±0.05ab	2.87±0.04d	3.92±0.08de	3.88±0.08e	45.25±1.26b	45.79±0.67b	8.66±0.08d	8.48±0.06d
	Hydro-P	91.89± 2.41b	94.17±4.42 b	3.98±0.12a	3.25±0.12c	4.74±0.07b	4.24±0.09cd	45.18±1.17b	44.88±0.81bc	10.49±0.12b	8.52±0.08d
	CaCl2-P	106.0 ±2.31a	106.90±2.99a	3.72±0.09ab	3.52±0.13bc	5.15±0.17a	4.52±0.13bc	46.36±0.93b	52.99±1.32a	11.11±0.15a	9.45±0.04c
	SA-P	111.6±2.33a	107.33±4.98b	3.85±0.04a	3.47±0.09bc	4.79±0.10b	3.99±0.06de	45.67±1.23b	±42.01±0.60c	10.50±0.17b	9.50±0.16c
Late Sowing	Control	31.33±4.15cd	19.33±4.26e	2.31±0.07bc	2.01±0.18c	1.35±0.05d	1.00±0.06e	40.43±0.43c	29.98±1.30de	3.34±0.13d	3.21±0.27d
	Hydro-P	42.0±3.79ab	35.0±2.65bc	2.90±0.16a	2.57±0.15ab	2.11±0.03b	1.13±0.05e	61.55±1.78a	23.27±1.44f	3.44±0.14d	4.85±0.12d
	$CaCl_2$-P	26.00±1.53de	31.7±1.76cd	2.55±0.8ab	2.78±0.09a	2.18±0.05b	1.46±0.05e	42.68±0.79bc	27.25±1.19e	5.10±0.07ab	5.37±0.15a
	SA-P	45.67±2.03a	49.7±4.80e	2.67±0.10ab	2.47±0.05a	2.36±0.06a	1.57±0.03e	44.76±1.23b	31.59±1.67d	5.28±0.05ab	4.98±0.20bc
LSD (≤0.05)		9.8466		3.0573		3.0236		3.4047		0.3499	

Mean of each treatment followed by various alphabets were statistically significant at $p\leq0.05$ under both sowing conditions (Duncan test, $p<0.05$).
Abbreviations: Hydro-P = Hydropriming; $CaCl_2$-P = Calcium chloride priming; SA-P = Salicylic acid priming

Table 3. Effect of seed priming on concentration of osmo-protectants of both wheat varieties (Sarsabz and Khirman) under Normal and Late sowing.

Treatment details		Proline (µmol g^{-1})		Glycine Betaine (µg g^{-1})		Total Soluble Sugars (mgg^{-1})		Total Phenolics (mg g^{-1})	
		Sarsabz	Khirman	Sarsabz	Khirman	Sarsabz	Khirman	Sarsabz	Khirman
Normal Sowing	Control	27.31±0.94e	29.06±0.77e	263.5±6.21e	496.1±9.88c	6.85±0.104c	6.09±0.15d	44.06±1.71g	60.87±1.56f
	Hydro-P	58.21±0.59b	47.72±1.38c	267.2±11.61e	799.5±15.18a	11.21±0.31b	12.70±0.11a	74.93±1.78d	83.91±1.89c
	$CaCl_2$-P	56.92±0.88b	41.23±1.13d	356.3±10.60d	693.0±15.81a	12.76±0.22a	11.41±0.25b	66.52±1.40e	105.80±1.38a
	SA-P	63.99±0.54a	57.78±1.10b	520.7±19.79c	532.5±9.94c	13.11±0.27a	12.96±0.19a	97.25±1.53b	78.70±1.90d
Late Sowing	Control	21.27±1.15e	21.24±1.00e	332.7±7.86f	440.3±14.67e	4.43±0.30d	4.57±0.18d	55.65±1.15e	49.42±1.38f
	Hydro-P	39.22±1.16bc	34.48±1.06e	560.0±13.84bc	978.5±12.61a	8.85±0.17ab	9.32±0.16a	83.77±0.77bc	66.52±1.40d
	$CaCl_2$-P	37.05±1.59cd	42.31±0.69ab	510.4±17.83d	520.8±17.94d	7.18±0.09c	8.40±0.23b	70.29±1.89d	88.55±1.67b
	SA-P	44.69±0.63a	38.26±0.67c	437.9±11.97e	583.1±11.04b	8.30±0.15b	9.12±0.17a	97.10±1.64a	80.73±2.34c
LSD (≤0.05)		2.9792		40.7380		0.5677		5.0394	

Mean value followed by various alphabets represents significant differences (LSD test, $p<0.05$) between various treatments under both sowing conditions (Duncan test, $p<0.05$).
Abbreviations: Hydro-P = Hydropriming; $CaCl_2$-P = Calcium chloride priming; SA-P = Salicylic acid priming

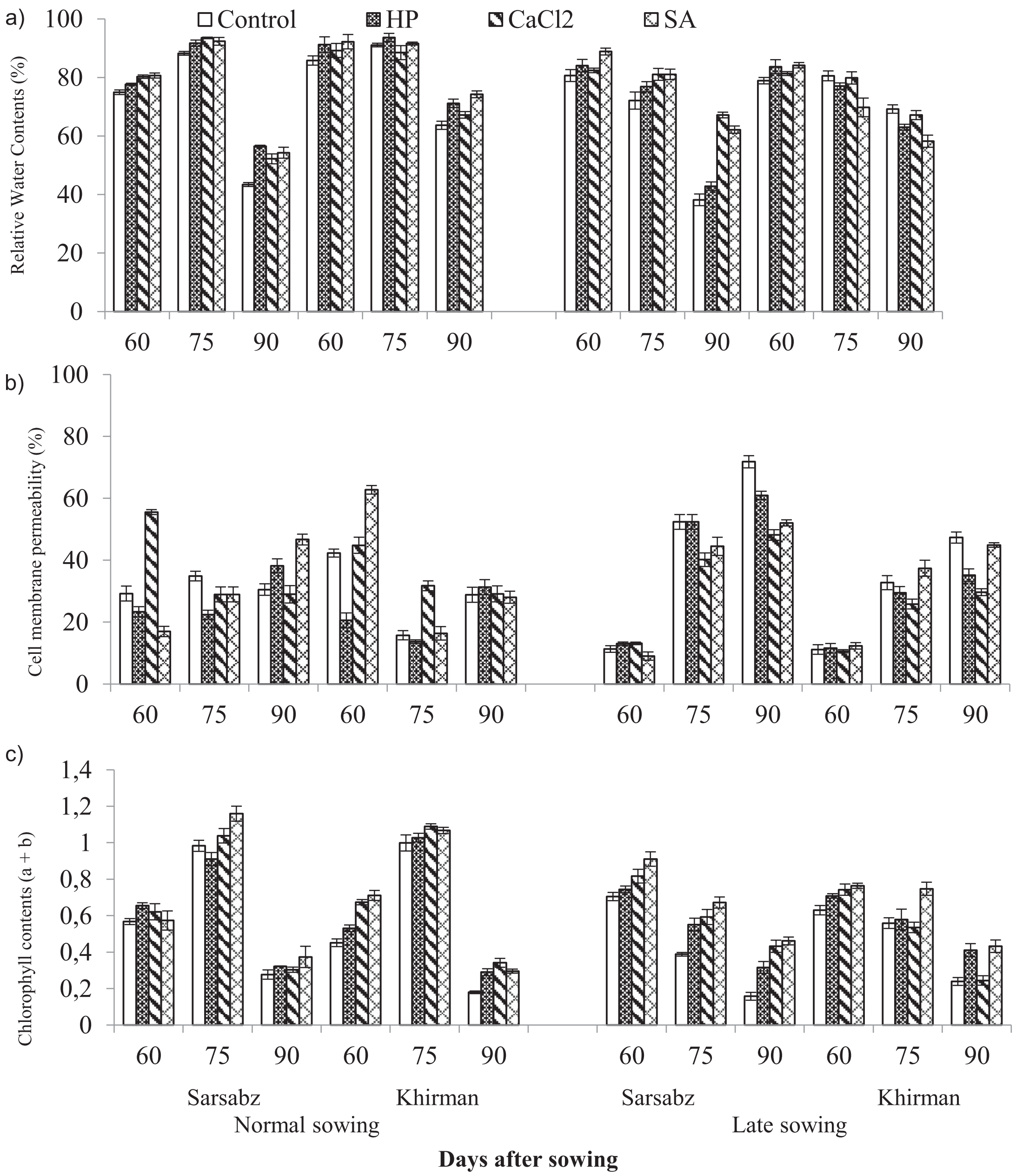

Fig. 3. Effects of seed priming treatments on relative water contents a), % cell membrane permeability b) and chlorophyll contents c) of both Sarsabz and Khirman wheat varieties at various growth stages (60, 75 and 90 DAS) under normal and late sowing.

photosystem-II (PS-II) reaction center. Decrease in photosynthetic pigments was due to thermal degradation of chloroplast. PS-II of winter crops like wheat is more susceptible to thermal stress as compared to summer season crops like rice due to seasonal adaptations. Maximum chlorophyll was analyzed at 75-DAS in both varieties under the influence of all treatments in normal sowing while these pigments were degraded at higher temperatures in late sown crop with maximum contents at 60-DAS. Sarsabz primed with SA exhibited highest photosynthetic pigments at 75-DAS and 60-DAS under normal and delayed planting, respectively (Fig. 3). Major reason behind higher initial chlorophyll contents was higher crop growth rates by early emergence and seedling stand establishment through seed priming under favorable weather conditions. However, leaf senescence occurred under elevated temperatures at later growth stages resulted in chlorophyll degradation [46].

Plants have a great potential to modify their metabolic activities against any change. Many cellular responses involving accumulation of various osmolytes are considered as adaptive mechanisms against high temperatures [47]. In this experiment, seed treatments partially reduced negative effects of heat stress by enhanced accumulation of proline, TSS, GB and TPC [48, 49]. Maximum proline was determined in SA treatments with 134.8% and 57.78% relative increase in Sarsabz and Khirman under normal conditions, respectively. Whereas SA-priming followed by hydropriming showed good proline concentrations in Sarsabz, while $CaCl_2$ treated Khirman showed highest proline with 9.99% relative increase under late sowing. SA acts as an endogenous signal molecule in inducing protection against abiotic stresses in plants while Ca^{2+} concentration is increased in cytosol by sudden exposure of stress, which triggers multiple biochemical changes due to Ca^{2+} binding on calcium mediated proteins. Highest TSS was measured in both varieties by SA-priming as well as in hydro-primed Khirman under normal conditions. Hydro-priming exhibited significant increment in GB in Khirman under both sowings. TPC were maximally augmented in Khirman by $CaCl_2$ followed by SA-primed Sarsabz under normal sowing while SA treatment of Sarsabz constituted better phenolic compounds under delayed sowing (Table 3). Among various seed priming agents, SA and $CaCl_2$ accelerated the accumulation of these protective metabolites under late sowing for partial protection against high temperature. These molecules including proline, soluble sugars, glycine-betaine etc. provide significant protection to plants against abiotic stresses and many studies reported effectiveness of exogenously applied SA in production of these osmo-protectants [50].

Conclusion

In the current study, the results indicated the improvement of both the varieties of the wheat under optimal and late sown conditions. it is noted that time reduced to 50% in their emergence significantly when treated with Salicylic Acid (SA) and $CaCl_2$. While high rates of emergence Index (EI) in both the Varieties of wheat i.e., Sarsabz and Khirman with reduced Mean Emergence Time (MET) with SA Priming. In case of leaf area, productive tillers and No. of tillers, SA and $CaCl_2$ priming contributed to more productivity. In growth parameters, higher yield of the wheat crop was observed in SA priming followed by the others. Seed priming also found helpful in increasing the RWC and played vital role in maintain the cell integrity after hydropriming. Moreover, high amounts of the chlorophyll contents, total phenolics proline and glycine betaine was observed, when seeds were treated with various priming techniques. Overall, this research concluded that wheat grown late in high temperature areas of Pakistan should give such priming treatments at large scale to improve the yield of the wheat crop an to abandon its present scenario of wheat shortage. This could help to enhance the productivity of the crop in late sown areas with drought conditions.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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