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# Response of Different Wheat (*Triticum aestivum* L.) Genotypes to Drought Stress Induced by Polyethylene Glycol-6000 during Seed Germination and Early Seedling Growth

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## Abstract

Drought is a serious challenge confronted by wheat farmers in Lesotho which can be mitigated by an introduction of tolerant wheat genotypes. The study was conducted under laboratory conditions at the National University of Lesotho with the objectives of (1) identifying wheat genotypes tolerant to drought stress, (2) ranking wheat genotypes according to their tolerance to drought stress. Factorial Design was laid-out using Complete Randomized Design with genotypes as factor A and PGE-6000 concentrations as factor B. Each treatment within a factor was replicated thrice in the laboratory. Four concentrations of PEG 6000; 0 bars, -0.5 bars, -1.0 bars and -1.5 bars simulated severity of drought to the growing seedlings. Seedling parameters such as root length, coleoptile length, plumule length, root fresh weight, coleoptiles fresh weight, plumule fresh weight and germination percentage were measured from 0 to 14 days. Data generated were subjected to ANOVA and genotype ranking using Genstat. Results revealed significant difference (P>0.05) among the genotypes responses to drought stress, different PEG-6000 concentrations and their interactions. As the concentration of PEG-6000 was increased, coleoptile length, radical fresh weight and plumule length decreased in all genotypes. PAN 3471, PAN 3368, PAN 3623 and SST 873 were the most tolerant genotypes to drought stress.

***Key words*****:** Drought tolerance, Polyethylene glycol-6000, *Triticum aesativum L.*, Lesotho

Wheat (*Triticum aesativum* L.) is a widely cultivated crop in the world ranking second to rice in terms of production and area grown [1], and it originated in south western Asia [2]. From this region, it spread and gained popularity world-wide through trade. The main wheat growing countries are China (134 million tons), India (98 million tons), Russia (85 million ton), United States (47 million tons), France (36 million ton), Australia (31 million tons), Canada (29 million tons) and Pakistan (26 million tons) [3]. Wheat production varies greatly in different countries and mostly depends on economic, managerial and environmental factors, namely; edaphic, rainfall, temperature and relative humidity. In Lesotho, wheat is grown in four agro-ecological regions though, high yields are obtained in the mountains followed by the foothills, and then lowlands [4]. For instance, in 2017-18 Agricultural year, total national wheat yield was 7,052 metric tons with Mokhotlong recording the highest production of 3,139 metric ton.

Drought is the major environmental stressor affecting wheat growth, development and ultimately yield [5]. This dramatically affects all key physiological processes, such as photosynthesis, respiration and uptake of mineral nutrients [6]. For instance, the plant may lose turgor as a result of water stress. This consequently leads to a reduction or cessation of growth by decreasing cell extensibility and cell expansion [7]. Wheat plant is able, to some extent, tolerate drought by effecting physical changes within its self frequently by creating signals for changing metabolism. Plants alter their metabolism in various ways in response to drought stress. It may respond to the stress by rolling their leaves to reduce the leaf surface area exposed to the sun [8] and the stomata may also be closed [9].

To relieve the plants from stress, proper care and management may be introduced and practiced effectively and efficiently. Water stress at any growth stage reduces yield [10]. Adequate soil water is critical for wheat production during the emergence, tillering, heading and grain filling stages [11]. The high and stable yields of wheat can only be achieved through irrigation [12]. Besides, tillage also plays a key role in changing the hydro-physical properties of the soil. Raised bed planting systems optimizes tillage operation, saves water, reduces lodging and ensures better fertilizer use [13].

Besides proper management, the tolerant wheat genotypes may also be used as these may help to obtain both high quality and wheat yield under drought conditions [14]. Several methods have been employed to identify the tolerant genotypes and efforts have been made in the past to screen different genotypes of wheat which differed in their tolerance to drought [15]. For instance, Ahmed [16] used a selection criterion for drought tolerant bread wheat genotypes at seedling stage where they were grown in a soil-based experiment. The genotypes were exposed to normal and drought conditions.

Many researchers working on induced drought studies have established protocol for identifying genotypes tolerant to

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drought using Polyethylene glycol (PEG)-6000. Polyethylene glycols (PEGs) are products of condensed ethylene oxide and water that can have various derivatives and functions. Since many PEG types are hydrophilic, they are favorably used as penetration enhancers [17]. PEG-6000 is a high molecular weight osmotic substance that has been used frequently as artificial abiotic stress inducer in many studies [18]. Hamayun *et al.* [19] indicated that, it is mainly used to evaluate tolerance of genotypes to drought during seed germination and establishment. The different levels of osmotic potential of PEG-6000 have been employed to maintain uniformity of plants in water accession along the experiment [20]. PEG provides a means of quantifying the water stress that roots experience therefore may be best used in screening of the tolerant genotypes.

Drought tolerance is the result of numerous morphological, anatomical and physiological characters, which interact with maintenance of growth and developmental processes under edaphic and climatic conditions [21]. This study was conducted with the objectives of: (1) identifying genotypes of wheat tolerant to drought stress, (2) ranking wheat genotypes according to their susceptibility and tolerance.

## MATERIALS AND METHODS

*Study area*

The study was conducted in the laboratory of the Department of Crop Science at the National University of Lesotho situated at Roma, 34 kilometers southeast of Maseru, the capital city of the kingdom of Lesotho. Its geographical coordinates are $29^0$ 27'$^{0}$" South, and $27^0$ 42'$^{0}$" East latitude. The Roma valley is broad and is surrounded by a barrier of mountains which provides magnificent scenery.

*Seed materials*

Forty-one wheat genotypes were collected from Department of Agricultural Research in Maseru, Lesotho. These genotypes were evaluated for yield and agronomic characters in four (4) agro-ecological zones of Lesotho, namely; lowlands, foothills, mountain and orange river valley. The names of the genotypes are SST 895, SST 875, Duzi, PAN 3541, SST 835, SST 8156, SST 843, PAN 3623, Krokodil, SST 886, PAN 3515, PAN 3400, SST 806, PAN 3644, PAN 3497, Sabie, PAN 3471, SST 8135, SST 884, SST 8154, PAN 3111, Senqu, Kougas, SST 356, Tugela DN, SST 3149, SST 317, PAN 3195, PAN 3161, SST 374, SST 347, Gariep, SST 316, Elands, SST 398, PAN 3368, Matlabas, Wedzi, Koonap, SST387, SST 387 and Kubetu.

*Experimental design*

The experiment was laid down in a factorial design with factor A being 41 wheat genotypes and factor B being four different drought stress levels. Each level within a factor was replicated thrice. The experimental units were arranged in a complete randomized design (CRD) with a total of 164 experimental units.

*Laboratory procedure*

Seeds of different wheat genotypes were surface sterilized by soaking them in 0.1% Sodium hypochlorite (w/v) for 2 minutes, after which ten seeds of each genotype was spread over a blotting paper in a petri-dish of 9 cm diameter. Each genotype was replicated three times. The petri-dishes were maintained in a growth chamber with bright diffused light, 70-80% relative humidity and temperature of 25-30 °C. An artificial drought stress was induced by preparing PEG-6000 solution having osmotic potentials of -1.0 bars following the procedure described by Kacem *et al.* [22], Lai *et al.* [23]. The solutions were prepared by dissolving different concentrations (0, -0.5, -1.0 and -1.5 bars) of PEG-6000 in 1000ml of distilled water. The different PEG-6000 concentrations were added to specific petri-dishes on daily basis to irrigate the seeds. The seeds were then incubated for 10 days in PEG-6000 solutions. The different concentrations simulated different levels of drought severity induced to wheat seeds. For the control condition, distilled and ionized water were used. The number of germinated seeds was recorded on daily basis.

*Data collection*

The number of germinated seeds was recorded on daily basis for10 days after sowing of seeds. At day 10, data on radical length, coleoptiles length, plumule length, radical fresh weight, coleoptiles fresh weight, and plumule fresh weight were collected.

*Data analysis*

Data generated were subjected to analysis of variance to establish the differences in a parameter among genotypes. Where F-values were significant ($p \leq 0.05$), the means were separated using Duncan Multiple Range test (DMRT). Ranking methods were performed to order genotypes on the basis of tolerance and susceptibility. Genstat 18 version (2016) statistical software was used for analysis of all statistical tools mentioned above.

## RESULTS AND DISCUSSION

*Analysis of variance for wheat genotypes' responses to drought stress*

The analysis of variance (Table 1) revealed significant differences ($P<0.05$) among wheat genotypes for germination percentage and root fresh weight. It also exhibited highly significant differences ($P<0.01$) among wheat genotypes for coleoptile length, plumule length, coleoptile fresh weight and plumule fresh weight. Root length, coleoptiles length and radical length were significantly affected by induced drought stress (PEG-6000), while plumule fresh weight, coleoptiles fresh weight and radical fresh weight were influenced by the cultivar. In general, all the parameters were affected by the interaction between wheat genotypes and PG-6000 concentrations.

*Variability in wheat genotypes' response to drought stress*

There were highly significant differences ($P \leq 0.01$) on germination percentage, plumule length, coleoptiles length and radical length among the genotypes, while a significant difference ($P \leq 0.05$ was revealed on radical fresh weight, coleoptiles fresh weight and plumule fresh weight (Table 2). Genotypes that managed to score 100% germination include SST 895, SST 8156 and SST 8154. The lowest germination percentage of 78.35% was experienced with Kubetu and Matlabas.

The longest radical lengths were obtained from PAN 3471, Sabie, PAN 3541, SST 806 with 22.03cm, 21.88cm, 21.77cm and 21.66, respectively. The shortest lengths were obtained from SST 8154, SST 316, Matlabas and Gariep with 16.83cm 15.14cm, 15.02cm and 12.50cm respectively. The highest radical fresh weight was recorded from Duzi, PAN 3541, Krokodil and SST 374. The lowest weights were observed from Gariep, SST 316, SST 356, SST 8135 and Wedzi. High coleoptile length readings were obtained from PAN 3368, Elands, Senqu, and SST 374. The low readings were measured from Duzi, SST 8154, SST 895 and SST 886. The maximum coleoptiles fresh weight values were recorded from SST 347, PAN 3368, Elands and Senqu. The minimum values were obtained from SST 843, SST 875, SST 8135, SST 8154 and Gariep. The plumule length was high on SST 874, PAN 3368, Koonap and Elands. The lowest values were measured from Gariep, Duzi, SST 886 and SST 884. The plumule fresh weights obtained from PAN 3541, Sabie, SST 374 and Tugela DN were high while the ones from SST 8154, SST 316 and SST 843 were low.

Table 1 Mean squares of wheat cultivar for various parameters

| Source of variation | Df | Mean square for wheat genotypes | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | GP % | RL | CL | PL | RFW | CFW | PFW |
| Genotypes | 40 | 1820.83* | 39.00** | 4.41** | 10.64** | 0.017* | O.01** | 0.01** |
| PEG Concentration | 3 | 382.13** | 192.74** | 6.65** | 66.51** | 0.04** | 0.02** | 0.09** |
| Cultivar × PEG concentration | 116 | 82.59* | 11.81** | 0.65** | 2.74** | 0.014** | 0.00** | 0.01* |
| Error | 330 | 76.84 | 5.91 | 0.14 | 0.87 | 0.12 | 0.00 | 0.01 |
| Total | 489 | 236.99 | 11.40 | 0.69 | 2.57 | 0.01 | 0.00 | 0.01 |
| GM | | 7.88 | 2.19 | 0.34 | 0.84 | 0.80 | 0.02 | 0.07 |

*Significant at P> 0.05, **Significant at P>0.01

GP= germination percentage, RL= radical length, CL= coleoptile length, PL= plumule length, RFW= radical fresh weight, CFW=coleoptiles fresh weight, PFW= plumule fresh weight

Table 2 Mean effects of genotypes on wheat germination parameters

| Genotypes | Parameter means and ranking of genotypes | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | RL | | CL | | PL | | RFW | | CFW | | PFW | |
| | Germination% | Rank | cm | Rank | cm | Rank | cm | Rank | cm | Rank | cm | Rank | cm | Rank |
| Duzi | 95.00 | 10 | 18.89 | 22 | 3.39 | 31 | 8.20 | 31 | 0.25 | 1 | 0.10 | 10 | 0.12 | 6 |
| Elands | 93.34 | 13 | 17.68 | 29 | 5.30 | 3 | 10.45 | 4 | 0.10 | 12 | 0.17 | 3 | 0.13 | 5 |
| Gariep | 26.72 | 25 | 12.50 | 34 | 4.04 | 16 | 7.01 | 36 | 0.07 | 15 | 0.11 | 9 | 0.10 | 8 |
| Koonap | 99.16 | 3 | 17.62 | 30 | 4.40 | 9 | 11.05 | 3 | 0.13 | 9 | 0.12 | 8 | 0.13 | 5 |
| Kougas | 96.65 | 7 | 19.15 | 20 | 4.08 | 14 | 10.14 | 5 | 0.18 | 5 | 0.13 | 7 | 0.14 | 4 |
| Krukodil | 95.84 | 9 | 20.24 | 12 | 3.82 | 22 | 8.63 | 12 | 0.21 | 3 | 0.11 | 9 | 0.11 | 7 |
| Kubetu | 78.35 | 23 | 19.73 | 13 | 4.27 | 10 | 9.24 | 16 | 0.11 | 11 | 0.14 | 6 | 0.13 | 5 |
| Matlabas | 78.35 | 23 | 15.02 | 32 | 4.83 | 5 | 9.60 | 13 | 0.11 | 11 | 0.15 | 5 | 0.12 | 6 |
| PAN 3111 | 96.12 | 8 | 18.64 | 25 | 3.51 | 31 | 8.65 | 22 | 0.12 | 10 | 0.12 | 8 | 0.15 | 3 |
| PAN 3161 | 96.67 | 6 | 19.04 | 21 | 3.93 | 16 | 8.68 | 23 | 0.15 | 7 | 0.16 | 4 | 0.15 | 3 |
| PAN 3195 | 88.33 | 18 | 18.28 | 27 | 3.81 | 24 | 7.70 | 34 | 0.14 | 8 | 0.13 | 7 | 0.13 | 5 |
| PAN 3368 | 99.16 | 2 | 19.60 | 14 | 5.38 | 2 | 11.95 | 1 | 0.15 | 7 | 0.18 | 2 | 0.16 | 2 |
| PAN 3400 | 96.68 | 5 | 18.64 | 25 | 3.83 | 21 | 7.95 | 32 | 0.16 | 6 | 0.12 | 8 | 0.13 | 5 |
| PAN 3471 | 94.18 | 11 | 22.03 | 1 | 3.87 | 23 | 9.44 | 14 | 0.15 | 7 | 0.13 | 7 | 0.13 | 5 |
| PAN 3497 | 95.84 | 9 | 20.36 | 11 | 3.93 | 17 | 9.72 | 10 | 0.13 | 9 | 0.12 | 8 | 0.13 | 5 |
| PAN 3515 | 96.67 | 6 | 17.52 | 31 | 3.65 | 26 | 8.38 | 25 | 0.13 | 9 | 0.12 | 8 | 0.12 | 6 |
| PAN 3541 | 95.00 | 10 | 21.77 | 3 | 4.07 | 15 | 8.74 | 21 | 0.22 | 2 | 0.16 | 4 | 0.16 | 2 |
| PAN 3623 | 93.35 | 12 | 20.62 | 9 | 4.10 | 12 | 10.03 | 6 | 0.13 | 9 | 0.14 | 6 | 0.15 | 3 |
| PAN 3644 | 97.50 | 4 | 19.21 | 19 | 3.58 | 28 | 8.95 | 19 | 0.10 | 12 | 0.10 | 10 | 0.12 | 6 |
| Sabie | 94.18 | 11 | 21.88 | 2 | 3.83 | 21 | 9.69 | 9 | 0.19 | 4 | 0.14 | 6 | 0.16 | 2 |
| Senqu | 88.35 | 17 | 17.70 | 28 | 5.11 | 4 | 10.18 | 5 | 0.15 | 7 | 0.16 | 4 | 0.15 | 3 |
| SST 3149 | 93.32 | 14 | 17.74 | 26 | 4.73 | 6 | 9.32 | 15 | 0.12 | 10 | 0.15 | 5 | 0.12 | 6 |
| SST 316 | 91.67 | 15 | 15.14 | 33 | 4.07 | 15 | 7.85 | 33 | 0.08 | 14 | 0.10 | 10 | 0.09 | 9 |
| SST 317 | 94.18 | 11 | 20.61 | 10 | 4.66 | 7 | 9.59 | 13 | 0.13 | 9 | 0.14 | 6 | 0.12 | 6 |
| SST 347 | 55.84 | 24 | 17.41 | 31 | 6.29 | 1 | 9.90 | 7 | 0.15 | 7 | 0.20 | 1 | 0.16 | 2 |
| SST 356 | 97.50 | 4 | 19.27 | 18 | 4.73 | 6 | 9.80 | 8 | 0.09 | 13 | 0.14 | 6 | 0.13 | 5 |
| SST 374 | 91.11 | 16 | 21.34 | 5 | 5.17 | 3 | 11.21 | 2 | 0.21 | 3 | 0.14 | 6 | 0.34 | 1 |
| SST 387 | 79.16 | 22 | 17.73 | 27 | 4.12 | 11 | 8.46 | 24 | 0.14 | 8 | 0.15 | 5 | 0.15 | 3 |
| SST 398 | 99.16 | 2 | 19.32 | 17 | 4.53 | 8 | 9.13 | 18 | 0.12 | 10 | 0.13 | 7 | 0.13 | 5 |
| SST 806 | 95.84 | 9 | 21.66 | 4 | 3.91 | 18 | 8.37 | 26 | 0.11 | 11 | 0.13 | 7 | 0.13 | 5 |
| SST 8135 | 85.01 | 19 | 18.85 | 24 | 3.86 | 19 | 9.67 | 11 | 0.09 | 13 | 0.10 | 10 | 0.11 | 7 |
| SST 8156 | 100.00 | 1 | 18.12 | 28 | 3.72 | 25 | 8.90 | 20 | 0.11 | 11 | 0.11 | 9 | 0.12 | 6 |
| SST 835 | 97.50 | 4 | 18.62 | 26 | 3.55 | 30 | 8.56 | 24 | 0.13 | 9 | 0.13 | 7 | 0.13 | 5 |
| SST 843 | 99.16 | 2 | 21.02 | 6 | 3.57 | 29 | 8.35 | 27 | 0.11 | 11 | 0.08 | 12 | 0.09 | 9 |
| SST 875 | 98.33 | 3 | 20.74 | 8 | 3.63 | 27 | 9.18 | 17 | 0.08 | 14 | 0.08 | 12 | 0.10 | 8 |
| SST 884 | 98.34 | 3 | 18.87 | 23 | 3.84 | 20 | 8.33 | 29 | 0.14 | 8 | 0.13 | 7 | 0.13 | 5 |
| SST 886 | 99.16 | 2 | 20.88 | 7 | 3.43 | 32 | 8.31 | 30 | 0.12 | 10 | 0.11 | 9 | 0.11 | 7 |
| SST 895 | 100.00 | 1 | 19.45 | 15 | 3.38 | 33 | 7.94 | 33 | 0.12 | 10 | 0.11 | 9 | 0.11 | 7 |
| SST 8154 | 100.00 | 1 | 16.83 | 32 | 3.20 | 34 | 7.60 | 35 | 0.12 | 10 | 0.09 | 11 | 0.08 | 10 |
| Tungela DN | 81.69 | 20 | 19.43 | 16 | 4.09 | 13 | 8.38 | 28 | 0.15 | 7 | 0.16 | 4 | 0.16 | 2 |
| Wedzi | 81.66 | 21 | 17.56 | 32 | 4.04 | 16 | 9.58 | 12 | 0.09 | 13 | 0.11 | 9 | 0.10 | 8 |
| LSD | 7.04 | | 1.95 | | 0.30 | | 0.71 | 37 | 0.07 | | 0.04 | | 0.07 | |

The results obtained from the different parameters revealed a great difference amongst the genotypes of wheat. Both PAN 3368 and Elands performed well on coleoptile length, coleoptiles fresh weight and plumule length with 5.38cm, 5.30cm, 0.18g, 0.17g and 11.95cm and 10.45cm, respectively. SST 8154 obtained the low values in radical length, coleoptiles fresh weight, plumule fresh weight, coleoptiles length with 16.83cm, 0.09g, 0.08g and 3.20g, respectively. Gariep also recorded low values of the radical length, radical fresh weight, coleoptiles fresh weight and plumule fresh weight with 12.50cm, 0.07g, 0.10g and 7.01cm, respectively. SST 347 showed high coleoptiles fresh weight and plumule fresh weight values of 0.20g and 0.16g, respectively. SST 843 exhibited the low values of both coleoptiles fresh weight (0.08g) and plumule fresh weight (0.09g).

The results on (Table 3) showed that SST 8154, SST 895 and SST 8156 were among the genotypes that appeared at the top ranking because they managed to score 100% germination, while Gariep, SST 347 and Kubetu appeared down the rank will low germination rate. PAN 3541 performed best in coleoptiles length, radical fresh weight and plumule fresh weight. SST 875 and SST 8135 recorded low radical fresh weight and coleoptile fresh weight. Sabie exhibited a better performance in both radical length and plumule fresh weight as it did not perform well in most measured parameters. SST 347 also performed well with the coleoptiles length of 6.29. SST 886 showed low values in coleoptiles length (3.43) and plumule length (8.31). The radical fresh weight of Duzi was high while coleoptiles length and plumule length were low. SST 316 also performed well in plumule fresh weight but the radical length and radical fresh weight were low. SST 806, Krokodil, and Koonap performed better in radical length, radical fresh weight and plumule length, respectively. These were all followed by Tungela DN, SST 884, SST 895, Wedzi, SST 856, and Matlabas which did not perform well in plumule fresh weight, plumule length, coleoptiles length, radical fresh weight and radical length.

*Analysis of variance for PEG- 6000 concentrations effect on germination and seedling growth*

Analysis of variance showed highly significant differences (P<0.01) among PEG-6000 concentrations for germination percentage, root length, coleoptile length, plumule length, root fresh weight, coleoptile fresh weight and plumule fresh weight (Table 3). The grand mean of germination percentage in all water stress levels and control were 90.66% and 92.40%, respectively. The highest germination percentage obtained with control was 94%, followed by -0.5 bars at 92.13%. The least germination percentage was obtained at -1.5 bars with 88.53%. The longest radical length measured in PEG-6000 was at -0.5bars with 20.54cm, followed by -1.0 bars with 19.30cm. The shortest length was recorded at -1.5 bars with 17.63cm. The greater weight in radical fresh weight was found in control where it had 0.15g. It was then followed by -0.5 bars with 0.14g. The least weight was obtained at -1.5 bars with 0.11g. The longest plumule length was obtained at the concentration of -0.5 bars with 9.77cm, followed by control which had 9.57cm. The shortest length of plumule measured was at -1.5 bars which had 8.08cm. The plumule fresh weight seemed to be greater at -0.5 bars where it got 0.16g, and then followed by control with 0.14g. The least weight was obtained at -1.5 bars with 0.10g. The coleoptiles length was high at -1.0 bars with 4.41cm which was followed by -0.5 bars with 4.17cm. The shortest length was recorded with control which had 3.88cm. The coleoptiles fresh weights were similar at the -1.0 bars and -0.5 bars which had 0.14cm. The control together with -1.5 bars had 0.12g (Table 4).

Table 3 Mean effects of water stress on wheat germination parameters

| Water stress (bars) | Parameters means and ranking | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Germination % | | RL | | CL | | PL | | RFW | | CFW | | PFW | |
| | | Rank | cm | Rank | cm | Rank | Cm | Rank | g | Rank | g | Rank | g | Rank |
| 0 | 92.40 | 1 | 18.42 | 3 | 3.88 | 4 | 9.57 | 2 | 0.15 | 1 | 0.12 | 2 | 0.14 | 2 |
| -0.5 | 92.13 | 2 | 20.54 | 1 | 4.17 | 2 | 9.77 | 1 | 0.14 | 2 | 0.14 | 1 | 0.16 | 1 |
| -1.0 | 91.02 | 3 | 19.30 | 2 | 4.41 | 1 | 9.11 | 3 | 0.13 | 3 | 0.14 | 1 | 0.13 | 3 |
| -1.5 | 88.53 | 4 | 17.63 | 4 | 4.10 | 3 | 8.08 | 4 | 0.11 | 4 | 0.12 | 2 | 0.10 | 4 |
| LSD | 2.23 | | 0.62 | | 0.10 | | 0.24 | | 0.23 | | 0.006 | | 0.21 | |

WS= water stress, RL= radical length, CL= coleoptile length, PL= plumule length, RFW= radical fresh weight, CFW=coleoptiles fresh weight, PFW= plumule fresh weight

*Analysis of variance for PEG concentrations X wheat genotypes interaction*

Analysis of variance (Table 1) showed the interaction between PEG-6000 concentrations and the genotypes to be significantly different (P<0.05) for germination percentage, plumule fresh weight and highly significant difference (P<0.01) for coleoptiles fresh weight, coleoptiles length, plumule length, radical fresh weight and radical length. At the lowest concentration levels of -1.5 bars, a drastic reduction of 88.50% in germination percentage was realized from (Table 4) of the results. The germination percentage in distilled water varied from 80% to 100% in all genotypes except Gariep which did not perform well in all the treatments, thus; 43% at 0g, 36.70% at -0.5 bars, 16.70% at -1.0 bars and 6.70% at -1.5 bars. The evidence from varying response of wheat genotypes against various water stress levels was also observed. It was observed that an increase in water stress led to a decrease in germination percentage. For instance, SST 317 at -0.5 bars had 96.70% germination. At -1.0 bars, germination percentage was 93.30% while at -1.5 bars 90% germination was obtained.

The responses of wheat genotypes against varying stress levels were different depending on each concentration. For instance, the longest radical length of 22.03cm was obtained from PAN 3471 while the shortest length was obtained from Gariep with12.50cm. At control, Senqu had the longest radical length of 22.10cm. Where PEG-6000 39g (-0.5 bars), 78g (-1.0 bars) and 117g (-1.5 bars) were applied, these genotypes had the longest radical length; SST 317 with 25.20cm, PAN 3497 with 23.2cm and SST 806 with 21.1cm, respectively. Radical fresh weight on (Table 3) indicated the highly significant difference among the wheat genotypes. Duzi obtained more weight than other genotypes with 0.25g while the lowest was Gariep with 0.07g from Table 3. From table 2 of the results, high significant difference on the coleoptiles length was noticed. The genotypes that managed to obtain the longest length include PAN 3368 and SST 8154 with 5.38cm and

3.20cm, respectively (Table 2). Coleoptiles fresh weight was high with SST 347 having 0.20g and SST 845 having 0.08g. PAN 3368 measured the longest length of 11.95cm while the shortest length was obtained of 7.01 cm on Gariep. Plumule fresh weight was high on SST 347 with 0.16g and the least was SST 8154 with 0.08g.

Table 4 Mean effects of water stress on germination parameters of wheat

| Water stress (bars) | Germination % | Plumule length | Coleoptile length | Radical length | Plumule fresh weight | Coleoptiles fresh weight | Radical fresh weight |
|---|---|---|---|---|---|---|---|
| 0 | 92.40 | 9.57 | 3.88 | 18.42 | 0.14 | 0.12 | 0.15 |
| -0.5 | 92.13 | 9.77 | 4.17 | 20.54 | 0.16 | 0.14 | 0.14 |
| -1.0. | 91.02 | 9.11 | 4.41 | 19.30 | 0.13 | 0.14 | 0.13 |
| -1.5 | 88.53 | 8.08 | 4.10 | 17.63 | 0.10 | 0.12 | 0.11 |
| LSD | 14.08 | 1.50 | 0.60 | 3.91 | 0.13 | 0.04 | 0.14 |

Table 5 Ranking of wheat genotypes for drought tolerance using -1.0 bars of stress

| Treatment | Parameter means and ranking | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | RL | | CL | | PL | | RFW | | CFW | | PFW | |
| | Germination % | Rank | cm | Rank | cm | Rank | cm | Rank | Grams | Rank | Grams | Rank | Grams | Rank |
| Duzi | 96.7 | 2 | 19.30 | 22 | 4.00 | 15 | 8.30 | 19 | 0.16 | 4 | 0.14 | 8 | 0.15 | 4 |
| Elands | 96.7 | 2 | 20.40 | 14 | 5.60 | 5 | 10.60 | 4 | 0.15 | 5 | 0.22 | 2 | 0.15 | 4 |
| Gariep | 6.7 | 11 | 4.00 | 34 | 1.40 | 22 | 2.10 | 23 | 0.03 | 13 | 0.05 | 15 | 0.04 | 11 |
| Koonap | 100 | 1 | 21.40 | 8 | 4.50 | 10 | 10.30 | 5 | 0.18 | 2 | 0.18 | 5 | 0.14 | 5 |
| Kougas | 100 | 1 | 19.00 | 23 | 3.90 | 16 | 11.70 | 1 | 0.12 | 7 | 0.11 | 12 | 0.16 | 3 |
| Krokodil | 93.3 | 3 | 19.80 | 18 | 4.30 | 12 | 9.30 | 11 | 0.16 | 4 | 0.13 | 9 | 0.10 | 9 |
| Kubetu | 73.3 | 9 | 19.90 | 17 | 4.30 | 12 | 9.20 | 12 | 0.13 | 6 | 0.16 | 7 | 0.13 | 6 |
| Matlabas | 86.7 | 5 | 14.90 | 32 | 4.80 | 8 | 8.80 | 15 | 0.12 | 7 | 0.18 | 5 | 0.11 | 8 |
| PAN 3111 | 93.3 | 3 | 19.70 | 19 | 3.60 | 18 | 8.80 | 15 | 0.11 | 8 | 0.10 | 13 | 0.12 | 7 |
| PAN 3161 | 93.3 | 3 | 19.40 | 21 | 4.10 | 14 | 8.30 | 19 | 0.19 | 1 | 0.18 | 5 | 0.15 | 4 |
| PAN 3195 | 83.3 | 6 | 18.30 | 24 | 4.90 | 7 | 8.30 | 19 | 0.10 | 9 | 0.14 | 8 | 0.11 | 8 |
| PAN 3368 | 100 | 1 | 19.60 | 20 | 6.30 | 2 | 10.90 | 2 | 0.12 | 7 | 0.20 | 3 | 0.13 | 6 |
| PAN 3400 | 100 | 1 | 21.10 | 10 | 4.30 | 12 | 9.00 | 13 | 0.13 | 6 | 0.12 | 10 | 0.13 | 6 |
| PAN 3497 | 93.3 | 3 | 23.20 | 2 | 4.20 | 13 | 9.50 | 9 | 0.14 | 5 | 0.14 | 8 | 0.14 | 5 |
| PAN 3515 | 100 | 1 | 16.30 | 30 | 3.90 | 16 | 9.00 | 13 | 0.12 | 7 | 0.11 | 12 | 0.12 | 7 |
| PAN 3541 | 93.3 | 3 | 23.00 | 4 | 4.30 | 12 | 9.30 | 11 | 0.19 | 1 | 0.17 | 6 | 0.17 | 2 |
| PAN 3623 | 96.7 | 2 | 20.20 | 15 | 4.20 | 13 | 10.00 | 6 | 0.15 | 4 | 0.15 | 7 | 0.16 | 3 |
| PAN 3644 | 100 | 1 | 20.70 | 12 | 3.80 | 17 | 8.70 | 16 | 0.10 | 9 | 0.10 | 13 | 0.11 | 8 |
| PAN3471 | 86.7 | 5 | 23.10 | 3 | 4.20 | 13 | 9.30 | 11 | 0.13 | 6 | 0.13 | 9 | 0.14 | 5 |
| Sabie | 90 | 4 | 24.40 | 1 | 4.20 | 13 | 9.50 | 9 | 0.13 | 6 | 0.15 | 7 | 0.15 | 4 |
| Senqu | 86.7 | 5 | 17.20 | 28 | 6.00 | 3 | 9.50 | 9 | 0.14 | 5 | 0.19 | 4 | 0.12 | 7 |
| SST 3149 | 93.3 | 3 | 17.30 | 27 | 5.00 | 6 | 9.70 | 8 | 0.12 | 7 | 0.17 | 6 | 0.10 | 9 |
| SST 316 | 96.7 | 2 | 18.10 | 26 | 4.70 | 9 | 8.90 | 14 | 0.08 | 11 | 0.14 | 8 | 0.10 | 9 |
| SST 317 | 93.3 | 3 | 21.50 | 7 | 5.60 | 5 | 10.80 | 3 | 0.14 | 5 | 0.18 | 5 | 0.15 | 4 |
| SST 347 | 50 | 10 | 18.20 | 25 | 6.90 | 1 | 8.70 | 16 | 0.17 | 3 | 0.24 | 1 | 0.17 | 2 |
| SST 356 | 100 | 1 | 20.60 | 13 | 4.30 | 12 | 9.40 | 10 | 0.09 | 10 | 0.12 | 11 | 0.11 | 8 |
| SST 374 | 90 | 4 | 20.60 | 13 | 5.90 | 4 | 10.90 | 2 | 0.12 | 7 | 0.15 | 7 | 0.15 | 4 |
| SST 387 | 76.7 | 8 | 19.70 | 19 | 3.90 | 16 | 8.50 | 18 | 0.11 | 8 | 0.15 | 7 | 0.17 | 2 |
| SST 398 | 100 | 1 | 18.30 | 24 | 4.40 | 11 | 8.00 | 21 | 0.13 | 6 | 0.13 | 9 | 0.09 | 10 |
| SST 806 | 100 | 1 | 20.00 | 16 | 5.00 | 6 | 9.70 | 8 | 0.12 | 7 | 0.15 | 7 | 0.15 | 4 |
| SST 8135 | 93.3 | 3 | 20.20 | 15 | 4.40 | 11 | 9.90 | 7 | 0.11 | 8 | 0.14 | 8 | 0.11 | 8 |
| SST 8154 | 100 | 1 | 16.30 | 30 | 4.00 | 15 | 8.70 | 16 | 0.12 | 7 | 0.10 | 13 | 0.11 | 8 |
| SST 8156 | 100 | 1 | 15.50 | 31 | 4.00 | 15 | 8.70 | 16 | 0.13 | 6 | 0.13 | 9 | 0.11 | 8 |
| SST 835 | 100 | 1 | 20.40 | 14 | 3.50 | 19 | 7.70 | 22 | 0.13 | 6 | 0.13 | 9 | 0.09 | 10 |
| SST 843 | 100 | 1 | 21.30 | 9 | 3.90 | 16 | 8.20 | 20 | 0.13 | 6 | 0.10 | 13 | 0.10 | 9 |
| SST 866 | 100 | 2 | 21.00 | 11 | 3.20 | 21 | 8.60 | 17 | 0.13 | 6 | 0.09 | 14 | 0.11 | 8 |
| SST 875 | 100 | 1 | 22.80 | 6 | 4.40 | 11 | 9.90 | 7 | 0.12 | 7 | 0.12 | 10 | 0.14 | 5 |
| SST 884 | 100 | 1 | 12.20 | 33 | 4.20 | 13 | 8.50 | 18 | 0.12 | 7 | 0.13 | 0 | 0.12 | 7 |
| SST 895 | 100 | 1 | 22.90 | 5 | 3.40 | 20 | 9.20 | 12 | 0.11 | 8 | 0.11 | 12 | 0.10 | 9 |
| Tugela DN | 80 | 7 | 19.70 | 19 | 4.30 | 12 | 8.50 | 18 | 0.17 | 3 | 0.18 | 5 | 0.18 | 1 |
| Wedzi | 83.3 | 6 | 16.70 | 29 | 4.20 | 13 | 9.00 | 13 | 0.04 | 12 | 0.14 | 8 | 0.12 | 7 |

RL= radical length, CL= coleoptile length, PL= plumule length, RFW= radical fresh weight, CFW=coleoptiles fresh weight, PFW= plumule fresh weight

*Ranking of genotypes for their response to drought stress*

Among the PEG-6000 concentrations used, the researchers mostly considered -1.0 bars (78g) as the best one to identify and select the best genotypes that may be used under drought stress. From (Table 5) of the results, Sabie and PAN 3497 had the longest radicle length of 24.40 cm and 23.20 cm respectively, while the shortest length was obtained from Gariep and SST 884 by 4.00 cm and 12.20 cm, respectively. Coleoptiles length was longer on SST 347 with 6.90 cm and Senqu with 6.00 cm. The shortest length was measured from Gariep and SST 866 with 1.40 cm and 3.20 cm, respectively. Kougas and PAN 3368 on plumule length had the greatest lengths of 11.70 cm and 10.9cm. Gariep and SST 866 had the shortest lengths of 2.10 cm and 8.20 cm, respectively. Coleoptiles fresh weight was high on Elands with 0.22g and Senqu with 0.19g. The least weight was of SST 866 with 0.09g and Gariep with 0.05g. PAN 3541 and PAN 3561 had the highest weight of 0.19g and the smallest weights were measured from SST 356 and Gariep with 0.09g and 0.03g, respectively. Plumule fresh weight was more on Tugela DN with 0.18g and PAN 3541 with 0.17g the least weight was obtained from SST 398 with 0.09g and Gariep with 0.04g.

The genotypes were ranked according to the tolerance of drought stress. On each parameter, the genotypes were designated 1 to 41 according to the manner in which they followed each other based on their tolerance and susceptibility. The genotypes that appear at the top were considered tolerant while those that apper at the bottom of the rank were considered susceptible. SST 884, SST 806 and SST 356 were among the genotypes that had 100% germination and they appeared at the top of the rank while Gariep, SST 347 and kubetu, respectively, had low germination rate and appeared on the lower rank.

*Variability in wheat genotypes*

The genotypes were exposed to different stress levels where they responded differently to four levels of drought stress induced by PEG-6000 concentrations as measured by germination percentage, radical length, coleoptile length, plumule length, radical fresh weight, coleoptiles fresh weight and plumule fresh weight. From ranking, the genotypes that had high germination rate and the longest root length were from the irrigated group while the dryland genotypes had low germination rate and the shortest root length. Coleoptile length, plumule length, radical fresh weight, coleoptiles fresh weight and plumule fresh weight measured high with the dryland genotypes that appear at the top ranking (Table 3). The difference in the response was brought about by genetic make-up of each genotype. Some genotypes were highly susceptible while others were highly tolerant to induced drought stress. Besides, there were some that were moderate. The most tolerant genotypes could be used when drought prevails so that a substantial yield can be obtained. The ranking of genotypes to drought tolerance can be correlated with severity of drought because it gives a clear picture onto which varieties may be able to tolerate stress, and at which level.

Crop plasticity to drought stress adaptation varies within genera, species and even genotypes [24]. The main morphological changes under drought conditions are root and shoot growth reduction [25]. The varied values were obtained from different wheat genotypes as opposed to different parameters measured under induced drought stress. Water deficit conditions significantly decreased germination percentage and plumule fresh weight. The largest decrease in germination percentage was observed in Gariep (26.72%), SST 347 (55.84%), Matlabas and Kubetu with (78.38%) while in plumule fresh weight, Gariep was still the lowest with 7.01g, followed by Duzi (8.20g) and SST 886 with 8.31g. However, some genotypes performed better on several parameters. For instance, Senqu showed good results on radical length, coleoptiles length, coleoptiles fresh weight and plumule fresh weight. SST 3368 performed well in coleoptile length, coleoptile fresh weight and plumule length. According to the results, the genotypes may be categorized into their susceptibility and tolerance to drought stress and this is consistent with other studies [26-27]. The results showed that the wheat genotypess studied varied greatly on susceptibility and tolerance to induced drought.

*PEG- 6000 concentrations*

Drought is the major threat in crop production. The water deficit results in the reduction of seedling growth, which is a critical factor for crop productivity [28]. The exposure of crops to drought stress affected most of the processes. The stomatal closure is considered as an early plant response to drought that allows relative water maintenance [29] and consequently reduces photosynthesis [30].

The results of this study indicated a decrease in germination percentage with water deficit. At control, the germination percentage was high. It was followed by -0.5 bars and -0.1 bars and the least germination percentage was -1.15 bars. The results were consistent with Datta *et al.* [31] who assessed the tolerance of wheat genotypes under laboratory conditions and observed that there was a declining pattern in water uptake by seeds. High water deficit might hamper the process of water uptake by seeds and thereby inhibiting the process of seed germination because the enzymes and hormones excretion may consequently be disordered [32]. Seed germination is a mechanism, in which morphological and physiological alterations result in activation of the embryo [33]. Before germination, seed imbibes water which will make it swell and activate enzymes. The enzymes will in turn act upon stored compounds to release energy required to trigger embryonic growth, consisting of radicle and plumule. This is a sensitive stage for water deficit, hence seed and seedling parameters are used as indicators for water deficit tolerance and susceptibility. Seed germination is a complex process that begins with water uptake and ends up with radical emergence from the surrounding tissue [34].

Radical length and plumule length were long at -0.5 bars and decreased with an increase in water stress. The shortest lengths were found at -1.5 bars. Similar results were found by Gravandi [35] when examining the different NaCl concentrations on germination, radical length and plumule length of clover genotypes where the relevant traits decreased with the increase in salt concentrations. The results are also supported by the findings of Ahmed *et al.* [36]. However, this may be an adaptive strategy of seed to prevent germination under stressful environment for ensuring proper establishment of seeds. Drought stress effects decrease with an increase in root growth rate in comparison with the growth of the aerial parts of the plant [37]. The reduction in longitudinal growth of shoot and root length growth are primary mechanisms in the face of stress. It is evident that, high root development under drought stress enables plant to reach deeper available water in the soil, hence survive to maturity [38]. Plumule fresh weight was also high at -0.5 bars.

Coleoptile is defined as the protective sheath covering the emerging shoot. It does not divide but increase in size as it accumulates more water. Studies show that having long coleoptiles improves seedling establishment under drought stress and is considered as a major factor in plant production [39]. The results of the present study showed the better performance of the genotypes in coleoptiles length and coleoptiles fresh weight where the genotypes were exposed to -1.0 bars. Similar results were also been reported by Alaei *et al.* [40] who was evaluating the germination characteristics of different durum wheat genotypes under osmotic stress. They discovered that the genotypes showing osmotic resistant may also be drought tolerance and may therefore survive drought stress with substantial production in terms of quality and yield.

*Wheat genotypes and PEG concentration interaction*

The results revealed the variation between drought stress levels and wheat genotypes. Variation was observed on: 1) the different induced drought levels (0, -0.5, -1.0 and -1.5 -bars), different responses of genotypes to different drought intensities and interaction of both stress levels and genotype. Significant interactions (P>0.005) revealed that genotypes responded variably to osmotic stress treatments, hence provided better opportunity to select drought tolerant genotypes at seedling growth stages [41].

The analysis of variance over four drought stress levels for genotypes under study revealed the presence of highly significant (P>0.01) differences for coleoptiles length, coleoptiles fresh weight, plumule length, radical length and radical fresh weight. Some parameters like germination percentage and plumule fresh weight were significant (P>0.05) and may be due to the genetic inheritance each genotype have. However, the expression of mean performance of all seedling traits was higher in control than in the osmotic stress. Radicle length, plumule length, radical fresh weight and coleoptiles fresh weight increased with increasing the stress level and these was in accordance with [42] who was screening 40 wheat genotypes on drought stress using different drought stress levels. The tolerant wheat lines were selected at seed germination where factorial experiments in a randomized complete block design with three replications were run at the Agricultural Research Center in Tehran. The results showed that all traits of drought stress significantly reduced. With increasing stress, most traits were reduced, the minimum impact of drought on root to shoot ratio and root dry weight were the highly affected. The genotypes were affected by moisture stress induced by PEG-6000.

*Ranking of wheat genotypes according to stress tolerance at -1.0 bars*

The estimated values of drought tolerance indices indicated that the identification of drought tolerant genotypes based on a single criterion was inadequate. Different indices better help to confirm drought tolerant in different genotypes. To determine the most desirable drought tolerant genotypes according to all the indices, mean rank of all drought tolerance criteria was used to order genotypes. In consideration to all indices, Sabie, PAN 3471, PAN 3541, SST 895, SST 875, and SST 317 were tolerant while Gariep, SST 884, and Matlabas were the most susceptible varieties. Increasing drought stress levels caused delay in seedling emergence. According to Ali and Elozeiri [43], decrease in seed germination under stress conditions is due to some metabolic disorders [44-45].

## CONCLUSION

The ranges in mean values of the treatments were variable. This is probably due to significant depression in traits due to PEG-6000 desiccation. The results further showed that maximum decrease occurred in root length and minimum in germination percentage. However, data of individual genotypes in osmotic stress indicated a little decrease in seed germination in more than half of the genotypes. Therefore, seed germination percentage alone cannot be used as a criterion for osmotic stress tolerance because it could not obviously discriminate among the genotypes. Similar to the present results, noted significant decrease in radicle and plumule length. Longer coleoptile length helps seedlings to emerge from the soil even at low soil moisture levels, hence can play an important role in the establishment of plant stand. Based on the measured traits, radical length, coleoptiles length, and plumule length may be used as the effective measures used in identifying the tolerant wheat genotypes.

*Recommendations*

PAN 3471 and PAN 3623 managed to perform best in most of the parameters that were measured under drought stress. They qualify to be the best varieties that may be used during prevalence of drought stress. Elands, SST 374, PAN 3368 and Tugela DN are recommended for adoption though they do not appear at the top ranking but they performed best in most of the measured parameters and they are the rain-fed genotypes. There is a need to conduct a similar study using pot experiment to verify the laboratory analysis. That may also be followed by field experiment which is appropriate for the farmers but of the conditions similar to that theirs.

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