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Research article

# The most relevant drought-tolerant indices for selecting barley drought-tolerant genotypes

Mbarek Ben Naceur<sup>\*1</sup>, Hatem Cheikh-Mhamed<sup>2</sup>

<sup>1</sup> Carthage University, National Gene Bank of Tunisia, 1080, Cherguia-1, Tunisia
<sup>2</sup> Carthage University, National Agronomic Research Institute of Tunisia, Agronomy Department, 2040, Ariana, Tunisia

# Abstract

During its development cycle, lack of water is one of the factors reducing plant growth and yields, in the world's arid regions. The identification of indices that characterize the most tolerant genotypes to drought is very useful since it allows us to evaluate the tolerance of large varieties collections within a short and early stage. This study aimed to identify the most efficient drought tolerance indicators and evaluate, from the early stage of plant development, the germination parameters that would be correlated with drought tolerance in the field. If such correlations were identified, it would be possible to screen dozens of genotypes in the laboratory and identify the most tolerant ones before moving into the field. To attain this objective, two tests were carried out: The first one was realized in the laboratory to assess some germination parameters (germination rate, root length, root number, etc.) of sixteen North African barley genotypes (Algerians, Tunisians, and Egyptians) at the germination stage, under polyethylene glycol (PEG-6000) induced stress. The second test was carried out in the field to measure the grain yield of the same genotypes, under favorable and limited water conditions. The laboratory test revealed significant differences between root lengths (RL) of different genotypes within each water regime and between different treatments (control and PEG-6000 solution). The obtained result showed the superiority of most Egyptian genotypes, especially under stress conditions induced by PEG-6000. The field trial also showed significant differences in grain yields under both water regimes (stressful and non-stressful regimes) and pointed to the high performance of the majority of Egyptian genotypes. The calculated indices [(STI), (SSI), (YSI), and (TOL)] showed variable correlations depending on the index used and concluded that STI and YSI are the best indicators of drought tolerance compared to the others. Among the germination parameters, only the root length (RL) under PEG stress is positively correlated with grain yield, obtained under drought conditions in the field. Therefore, it would be possible to use this parameter to select, at an early stage, the most drought-tolerant genotypes.

Keywords: Barley; correlation; drought; polyethylene glycol; tolerance index; stress

### 1. Introduction

Drought is the main factor limiting agricultural productivity in many countries worldwide. It affects all aspects of plant growth and causes a series of changes affecting morphological, physiological, and biochemical plant characteristics related to the expression of drought tolerance genes (Gray and Brady, 2016; Gerszberg and Hnatuszko-Konka, 2017; Seleiman et al., 2021). According to Kuru (2023), plants undergo morphological changes that are critical to responding to water deficiency, such as a decrease in growth rate, an elongation of the root system, and an altered root-to-aerial part ratio.

Underwater scarcity conditions, the most significant root changes were the modification of their architecture, such as root suberization avoiding water loss, and allowing the plant to

\* Corresponding author. E-mail address: nour3alanour@yahoo.com (M. Ben Naceur). https://doi.org/10.51753/flsrt.1362571 Author contributions

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survive until the soil humidity becomes suitable again, and the increase in root hairs necessary for the absorption of moisture from the soil (Minocha et al., 2014; Hassan et al., 2023). Basu et al. (2016), stated that the formation of small roots under drought conditions was an adaptive pathway since it improves water absorption by providing a greater absorbent surface. Moreover, the presence of rhizodermal tissue, with thickened outer walls (suberized), or with reduced cortical layers, was also considered an adaptive pathway for drought survival. Other changes, in response to drought, have been reported such as root thickness or root\_thinner (Kou et al., 2022), and both responses are beneficial for crops subjected to drought. The biochemical changes expressed at the leaf level are related to the osmotic accumulation (proline, glycine betaine, soluble sugars etc.) to maintain the turgidity potential as high as possible and allow the plant to survive (Sakr et al., 2012; Cai and Gao, 2020; Huang et al., 2021). Yooyongwech et al. (2017), working on sweet potatoes also confirmed biochemical changes under drought conditions such as an increase in total soluble sugar in storage root tissues and an increase in proline and sucrose content in leaf tissues to maintain the leaf osmotic potential. In arid and semiarid regions of North Africa and sub-Saharan Africa, the main livelihood is based on rain-fed agriculture. These regions are characterized by irregular rainfall and frequent and intense droughts, which force farmers to put in place tools to deal with temporal water shortages. In this context, more, than 3/4 of Tunisia's surface area consists of semi-arid, arid, and desert regions. In this country, barley cultivation is rain-fed and covers an area varying from 500 to 600 ha out of 1500 ha of total cereals. The national average barley yield is only 0,08 kg/m<sup>2</sup>. This is mainly due to the recurring lack of precipitation and the inadequate technical package used. Thus, the selection of drought-tolerant barley cultivars in this country is of paramount importance to improve the yields of this crop and exploit potential untapped production areas.

Selecting the most drought-tolerant genotypes is difficult due to the unavailability of rapid and reproducible screening techniques (Hassan et al., 2023). Nevertheless, despite these complications, some studies were realized in different ways. Such as measuring the plant's relative water content, the plant cover temperature (Tembe et al., 2017), the osmoticum accumulation, the membrane integrity (Geetha et al., 2017; Mahdavi et al., 2023), the root system parameters (root length, root number, root diameter, and architecture) (Maiti, 2012; Lalić et al., 2017), and the yield components under stress conditions (EL-Shawy et al., 2017). According to Negisho et al. (2022) and Li et al. (2023), drought tolerance indices provide measures based on yield loss under drought conditions compared to that obtained under favorable conditions. Several authors (Ilker, 2011; Mohammadi et al., 2011; Ayranci et al., 2014; Gitore et al., 2021) defined stress tolerance (TOL) as the difference between the yield obtained under favorable conditions (Yp) and that obtained under stress conditions (Ys). For their part, Sánchez-Reinoso et al. (2020) recommended the stress susceptibility index (SSI) for assessing the sensitivity of genotypes in varying environments. Lamba et al. (2023) proposed a yield index (YI) and yield stability index (YSI) as an assessment of genotype stability under water deficit and favorable conditions. Gitore et al. (2021) used the Stress Tolerance Index (STI) to identify the most productive genotypes under favorable and those of water deficit. Other researchers have used other modified indices. Some of them have conceived new methods for monitoring drought-induced vegetation stress called the Vegetation Drought Response Index (Lamba et al., 2023; Yin and Zhang, 2023). Although none of the drought indices is necessarily better than the others, some indices are better suited for specific purposes than others (Karavitis et al., 2011).

This study focused on assessing the effectiveness of drought tolerance indices for the selection of drought-tolerant barley genotypes at the beginning (germination) and at the end (maturity) stage in the field. We were interested in germination parameters, grain yield, and indices previously cited and tried to identify correlations that may be useful for the identification of drought tolerant genotypes at an early stage.

### 2. Materials and methods

Sixteen barley genotypes were selected from three North African countries [Tunisia (5 genotypes), Algeria (5 genotypes), and Egypt (6 genotypes)]. This material is as follows: Kairouan (V4), Rihane (V7), Sidi-Bouzid (V8), Sabra (V9), Tombari (V10) from Tunisia, Techedrett (V15), Saïda (V17), Sidi-Mehdi (V18), Ras-El-mouche (V19), Naïlia (V20) from Algeria and Giza 123 (V23), El Arich (V24), Ksar (V25), Giza 2000 (V26), Giza 125 (V29) and Giza 131 (V30) from Egypt.

#### 2.1. Tests conduct

The first test was carried out in the laboratory where 20 barley seeds of each genotype were germinated in Petri dishes (90mm diameter), containing filter paper and distilled water (Control) or a polyethylene glycol solution (PEG-6000) at a 10% concentration (Stressed). Each treatment (Stressed or not) is repeated 4 times. The second trial was realized in the field with standard agronomic practices of barley crops during two successive growing seasons (2013-2014 and 2014-2015). The 16 barley genotypes were selected as part of the New Partnership for African Development (NEPAD) project, carried out in Algeria, Tunisia, and Egypt at the same time. The soil on which the tests were conducted, and the seedbed preparation actions have been previously described (Ben Naceur et al., 2018). The seed rate was calculated based on 250 seeds/m<sup>2</sup>.

The experiment was carried out using a randomized complete block design (RCBD) with four repetitions for each genotype per block. The area of each elementary plot was 4 m<sup>2</sup>  $(2m \times 2m)$ . The blocks are separated from each other by 2m while the elementary plots and the rows are separated by 0.50m and 0.20 m, respectively. The recorded climatic data through the two experimental growing seasons are shown in Fig. 1. They were obtained from the historical weather site. The comparison of monthly average temperature and precipitation during the two growing seasons (November to May) showed that average temperature values were very near (Fig. 1a), while precipitation in 2014 (349 mm) was much lower than in 2015 (421 mm) (Fig. 1b). If we compare the precipitation of the months during the two growing seasons, we can see a considerable difference in January and February rains with 29 and 18 mm in 2014 compared to 70 and 118 mm in 2015 (Fig. 1b).

### 2.2. Parameters measured

#### 2.2.1. Germination parameters

Germination was realized in a germination chamber (temperature: 25/18°C and 12h of light to speed growth) in Petri



Fig. 1. Comparison of monthly averages of temperature (a) and precipitation (b) during the two growing seasons (2014 and 2015).

dishes containing either distilled water (Control) or a 10% PEG-6000 solution (Stressed). Michel and Kaufmann (1973), established a relationship between the PEG-6000 concentration of a given solution and its osmotic potential:

 $\label{eq:phi} \begin{aligned} \pi = (\text{-} \ 0.0118 \times C) - (0.000118 \times C) + (0.000267 \times CT) + \\ 0.00000839 \times C_2T) \end{aligned}$ 

Where C is the PEG-6000 concentration and T is the temperature.

In this case, the osmotic potential of the solution used is - 1.48 bars.

After one week of cultivation in Petri dishes, the parameters measured were:

- The germination rates.
- The root numbers.
- The root length is expressed in cm.
- The Stress Tolerance Index (STI) is based on root length in the PEG-6000 solution.

### 2.2.2. Grain yield obtained in the field

The total grain yield (stressed or unstressed) is calculated after harvesting the elementary plots and expressing the results in  $kg/m^2$ .

#### 2.2.3. Drought tolerance indices

Several indices that describe drought tolerance defined by (Ayranci et al., 2014; EL-Shawy et al., 2017; Hellal et al., 2019; Sánchez-Reinoso et al., 2020; Li et al., 2023), were used in this study, in the germination test and, in the field trial:

The stress susceptibility index noted SSI: SSI = [1 - (Ys)/(Yp)]/SI

Stress intensity noted SI (Stress Intensity): SI = [1- (Ys)/(Yp)]

The stress tolerance index denoted STI (Stress Tolerance Index):

 $STI = [(Yp)X(Ys) / \bar{Y}p2]$ 

Stress tolerance noted TOL (Tolerance): TOL = (Yp - Ys)

The yield stability index noted YSI (Yield Stability Index): YSI = Ys/Yp

Where Ys and Yp are the yields of genotypes evaluated under stressful and non-stressful conditions and Ys and Yp are the averages of all genotypes evaluated under stress and favorable conditions.

### 2.3. Statistical analysis

The experiment was carried out using a randomized complete block design (RCBD) with four repetitions for each genotype per block. The area of each elementary plot was 4 m<sup>2</sup>. The sowing date was realized manually on 1 November for each growing season. The sowing density was 60 grains/linear meter. The first two blocks were conducted in the open field from the sowing date until harvest (Control) and the two-second blocks were also conducted in the open field from the sowing date (November) until the ear swelling and early heading stage (early March). From this date until harvesting, the plants were protected by a plastic film preventing rainfall and without irrigation (Stressed). The data collected during the two growing seasons were statistically analyzed using SAS software. Genotype means were compared using a Fisher's test by least significant difference at ( $P \le 0.05$ ).

### 3. Results and discussion

# 3.1. Germination rate, root number, and root length at the germination stage

The germination rate calculated in this study indicates the percentage of germinated grains out of the total number of grains placed in Petri dishes for germination. In this study, PEG-6000 as a stressor did not induce considerable change in the final germination of different barley genotypes. However, it brings a significant delay in germination without completely inhibiting it. This delay is common in all germination tests because the seeds need more time to absorb enough water and initiate germination. Although most seeds have germinated both under non-stressful and under PEG-6000 stressful conditions, the obtained seedlings under stressful conditions are not all viable (poorly developed roots). The same observation was noted for the root number. In fact, the PEG-6000-induced stress did not significantly influence the root number and although a slight reduction was observed under stress conditions, no significant

difference was noticed (Fig. 2). In contrast, some genotypes produced the same root number or sometimes more roots in stress conditions than in non-stress conditions (V8; V23; V25).

#### Table 1

Root length (RL) variation of 16-barley genotypes under stress (PEG-6000) and non-stressful conditions.

Genotypes	Non-stress conditions T0	PEG-6000 stress conditions. T10		
	Mean root length in cm	Mean root length in cm		
V4	5.97 fgh	4.08 abcd		
V7	6.58 bc	5.012 a		
V8	6.16 gh	4.11 abc		
V9	3.92 h	2.68 f		
V10	7.99 a	3.10 def		
V15	5.27 def	3.49 cdef		
V17	5.00 efgh	3.76 bcde		
V18	5.98 bcde	4.50 ab		
V19	4.79 fgh	3.64 bcdef		
V20	5.14 efg	3.54 bcdef		
V23	6.32 bcd	3.53 bcdef		
V24	5.07 efg	3.10 def		
V25	5.66 cdef	3.52 bcdef		
V26	6.89 ab	3.03 ef		
V29	7.94 a	4.05 abcd		
V30	6.41 bc	4.14 abc		

\*(Means with the *same letter* are *not* significantly *different* from each other)

Therefore, PEG-6000-induced stress did not significantly affect germination rate or root number, but it negatively influenced root length. This is why we focused on root length rather than on root number or on the germination rate itself (Table 1). This result is per those of Kou et al. (2022) who stated that water deficit induces an increase in root hair density, which in turn, increases the contact between the root surface and their environments. It is recognized that, under water-limited conditions, sensitive genotypes were incapable of uptake sufficient water due to their inability to emit deep roots in search of moisture, whereas tolerant genotypes produced extended roots to obtain water from the solution or the lower surface of the soil when they were sown in the field. Therefore, the root



Fig. 2. Barley root number at the germination stage.



Fig. 3. Stress Tolerance Index of barley genotypes based on root length under PEG-6000 conditions.

length character may be used, among others, as a reliable selection criterion for barley drought resistance. Table 1 revealed variability in the average root length both under the control and under PEG-6000 stress conditions. This parameter, statistically analyzed, had significant differences at the 5% threshold in stressful and non-stressful conditions.

The classification of root length in the Control showed eight different groups. The best performances are observed in the V10, V29, and V26 genotypes, which occupied the first cluster. Their root length varied between 6.89 and 7.99 cm while V4, V8, V17, V19, and V9 occupied the last group and exhibited the shortest root lengths that varied from 3.92 to\_6.16 cm. The other genotypes are intermediate. This result, expressing variability between genotypes, complied with those observed by Min et al. (2022) on maize and by Aslam et al. (2023) on cotton. Similarly, under the PEG-6000 conditions, the root length analysis showed significant differences at the 5% threshold and hierarchized the genotypes into six (6) groups where V4, V7, V8, V18, V29, and V30 occupied the first class with 4.05 to 5.012 cm, in length. On the other hand, the remaining genotypes showed the shortest root and therefore occupied the last class with lengths varying between 2.68 and 3.76 cm. Although the stress has reduced the extending root of the majority of the genotypes tested, some of them were able to maintain sufficiently extended roots. The ability of these barley genotypes to maintain growing roots under stress conditions, suggests the upholding of certain gene expressions involved in root elongation such as (Deeper Rooting 1 (DR01) as reported by Uga et al. (2013). These genes would promote the absorption of water and allow a correlative growth of these roots.

Other genes could increase the osmoticum level in the roots of the tolerant genotype exposed to drought and increase the activity of certain antioxidant enzymes (catalase, peroxidase, etc.) to reduce oxidative damage due to environmental stress, as demonstrated by Cai and Gao (2020) and Aslam et al. (2023) in recent studies. Therefore, root extension under stress conditions could inform us about the stress-tolerant genotypes and constitute an appreciable indicator of tolerance to water deficit. The stress tolerance index, based on the root length under favorable or under stress conditions (induced by PEG-6000), illustrated in Fig. 3 showed that V7, V30, and V29 genotypes were the most tolerant ones to stress, on the other hand, V9 was the most sensitive. Long roots, which were well anchored in the substrate, were a beneficial factor influencing the capacity of the plant to absorb water from the soil's deep layers. Based on the individual root or the whole root system, different parameters such as root length, diameter, or architectural patterns, have been used as potential indicators of stress tolerance in some previous case studies. In this context, Lalić et al. (2017) and Aslam et al. (2023) have used root elongation as an indicator of stress tolerance for barley and cotton, respectively. They suggested that the uptake of water is directly linked to root development and architecture, confirming the choice of this parameter (root length) as a criterion for evaluating plant tolerance to stress under PEG-6000.

# 3.2. Evaluation of the genotype's tolerance to drought in the field

## 3.2.1. The harvested grain yield

The grain yield was obtained after collecting the elementary plots and expressing the results in Q/ha. The result obtained showed that the Egyptian genotype V30 produced the best yield in both favorable and drought conditions (Table 2). This genotype produced 48 Q/ha (0.48kg/m<sup>2</sup>), underwater favorable conditions against the average of all the genotypes which was 35 Q/ha (0.35kg/m<sup>2</sup>), showing a superiority of about 37%, compared to the average. It also produced 46 Q/ha (0.46kg/m2) under water deficit conditions against the average of all genotypes tested which was 27.28 Q/ha (0.27kg/m2), that to say, an increase of 68.62% compared to the average of all genotypes. Abdel-Moneam et al. (2014) and Hellal et al. (2019) tested the same genotype (V30) in other geographically varied sites and showed its good yield and wide adaptation to different environments.

Statistical analysis (Table 2) classified all the genotypes into eight (8) groups in the control and five (5) groups in the stressed. Whatever, the water regime used, V30 and V7 produced the best yield and therefore occupied the first class while V10 produced the lowest yield. The high grain yield produced by the V30 and V7 genotypes confirms their tolerance to drought as compared to the remaining genotypes. The best performance of these two genotypes observed in both water regimes was consistent with what Hellal et al. (2019) found. These results were also in agreement with those of Abd El-Raouf et al. (2012) who compared the yield of several barley genotypes under drought conditions of which (V30) was among the most efficient.

# Table 2Indices measured.

Genotypes	Yp	Ys	TOL	SSI	STI	YSI
V30	48.05 a	43.27 a	4.79	0.429	1.646	0.900
V7	46.11 ab	42.45 a	3.66	0.342	1.550	0.921
V24	45.74 ab	31.93 b	13.80	1.300	1.156	0.698
V26	42.59 abc	34.27 b	5.74	0.841	1.155	0.805
V25	41.50 abcde	35.27 b	5.66	0.595	1.143	0.862
V29	40.92 abcd	33.83 b	7.66	0.795	1.111	0.815
V20	38.73 abcde	28.33 bc	10.40	1.1561	0.869	0.731
V18	37.41 bcdef	31.67 b	5.74	0.661	0.938	0.974
V15	35.84 bcdef	26.43 bc	9.417	1.130	0.750	0.737
V8	32.50 cdefg	21.63 de	10.87	1.440	0.557	0.675
V4	31.66 defg	23.97 cd	7.69	1.046	0.601	0.757
V17	30.77 efg	16.16 de	14.61	2.043	0.394	0.525
V23	27.29 fgh	17.16 de	10.13	1.598	0.371	0.629
V9	27.00 fgh	19.39 de	7.62	1.215	0.414	0.718
V19	23.10 gh	18 de	5.10	0.951	0.329	0.779
V10	19.43 h	12.8 e	6.63	2.044	0.197	0.659

\*(Means with the *same letter* are *not* significantly *different* from each other)

# 3.2.2. Evaluation of grain yield indices under water deficit conditions

The indices describing sensitivity (SSI) and stress tolerance (STI) are illustrated in Table 2, which always showed the superiority of production of V30 and V7 and the low yielding of V10whatever the water regime used. The high-stress tolerance index (STI) of these two genotypes during the two growing seasons indicated their adaptability to different humidity levels compared to other genotypes whose ranking changed according to the soil moisture conditions. The interaction (genotype x environment) which determined the yield stability (YSI) also revealed the yield superiority of these two genotypes in both cases of water regime. Our result displayed also other high-yielding genotypes (V26, V24, and V25) which could differ in terms of yield stability. This could occur when genotypes are only productive under favorable conditions or when the yield difference, in both water regimes, is small. Consequently, the V30 and V7 genotypes could be used as progenitors in varietal selection programs for drought resistance. Nevertheless, genotype classification based on TOL or SSI indices exhibited a slightly different trend than that generated by STI (Table 2), confirming the inability of SSI to differentiate drought-tolerant genotypes from those with low yield potential, as Li et al. (2023) have suggested. The highest tolerance index TOL values were obtained in V17, V24, V8, V20, and V23 genotypes (14.61; 13.80; 10.87; 10.40, and 10.13 respectively). Likewise, the highest values of the stress susceptibility index (SSI) were obtained in V10; V17; V23; V8, and V24 genotypes (2.044; 2.043; 1.60, 1.44, and 1.30, respectively) (Table 2). Most of these genotypes showed satisfactory yield under non-stressed conditions but low yield under drought conditions. This implies that choices based on high values of TOL and/or SSI would result in sensitive genotypes with low yields under drought conditions.

However, the lowest values of SSI or TOL (values  $\leq 1$ ) could also be practical indices characterizing the most tolerant genotypes, similar to STI or YSI, as proposed by Sánchez-Reinoso et al., (2020) for common bean genotypes subject to water deficit. Nevertheless, when the SSI index was low, it could indicate also that the production potential of the genotype is low and the genotype might not be productive under both water regimes. This observation was confirmed by Li et al. (2023) who used (SSI) as a screening criterion for drought resistance in wheat and revealed the inability of this criterion to distinguish between the most tolerant genotypes and those having low potential yield. Conversely, when STI and YSI indices were higher, the genotype might be productive, under stressful and non-stressful conditions. These results agreed with those of Hellal et al. (2019) on barley and Abdul-Mannan et al. (2023) on maize. Similar results to ours were reported by Mohammadi et al. (2011) indicating that (STI) index was well-appropriate for selecting the most productive RILs (Recombinant Inbred Lines) under two contrasting water regimes.

Moreover, Gitore et al. (2021), working on orange-fleshed sweet potato genotypes, reported that drought tolerance was indicated by genotypes with high Tolerance Index (STI) values. Also, Mahdavi et al. (2023) working on wheat, reported the same conclusion in which tolerant genotypes were characterized by a stress tolerance index and a yield stability index (STI and YSI) both high and indices (TOL and SSI) relatively low. It is therefore clear that the drought tolerance index (STI) is a strong discriminator between potentially water-stress tolerant genotypes and other high-yielding genotypes. However, the (SSI) and (TOL) indices provided usually variable results, which do not allow the selection of the most stress-tolerant genotypes. These two last parameters have already shown their limits in



Fig. 4. Correlation between (Ys) on one hand and (STI) and (YSI) on the other.

wheat (Anwar et al., 2011) and in beans (Asadi and Seyedi, 2021).

# 3.3. Correlations between stress indices and yields under drought conditions (Ys)

Positive correlations between grain yield under stress conditions (Ys) and the stress tolerance Index (STI) on the one hand, and between (Ys) and the yield stability index (YSI), on the other hand, have been established (Fig. 4). High (STI) and (YSI) values indicated that the genotypes are drought tolerant. Consequently, the Egyptian (V30) and Tunisian (V7) genotypes are the most efficient and well-adapted to normal and adverse



**Fig. 5.** Correlation between (Ys) and (SSI) indices for Tunisian (a), Algerian (b), Egyptian (c) varieties separately.

conditions. This result was consent with what Nouri et al. (2011) and Mahdavi et al. (2023) have reported on wheat. The obtained results are also similar to what Hellal et al. (2019) have obtained on barley in another geographical region different from ours. These authors showed also a positive and highly significant correlation between the stress tolerance index (STI) and grain yield under water stress\_conditions (YS) and stress stability index (YSI). They reported that these indices could discriminate groups of varieties that express superior indices of (STI and/or YSI) which are correlated with high yielding in stress conditions.

Similarly, the recorded data in this study showed that grain yield under stress conditions (YS) had a significantly negative correlation with the (TOL) and with the stress susceptibility index (SSI) for all genotypes (Fig. 5 a-c and Fig. 6.). Poudel et al. (2021) who were working under heat stress confirmed our result and revealed a negative and significant correlation between (SSI) and (Ys). They concluded that selection based on low SSI values would identify the most heat-stress-tolerant genotypes. However, when the SSI value exceeds 1, this index is no longer suitable for selecting the most stress-tolerant genotypes. These results agreed with those of Gitore et al. (2021) obtained on Orange Fleshed Sweet Potato and with that of Hellal et al. (2019) obtained on barley, which suggested that genotypes with the lowest (SSI) displayed drought resistance and those of high value of (SSI) are more susceptible to drought.



Fig. 6. Correlation between (Ys) and (TOL) index for all genotypes.

# 3.4. Correlation between yields under water deficit and germination parameters under PEG-6000-induced stress

Many plants are able to develop deep and extensive root systems under water-scarce conditions allowing them to draw water from deeper layers of the soil. This trait is of particular importance for crops, which often experience periods of water deficit. The illustration below (Fig. 7) showed a positive correlation between root length (RL), at the germination stage in PEG-6000, and grain yield (Ys) obtained in the field under drought conditions. The ability of barley genotypes to keep their root system growing despite the constraint imposed by PEG-6000 during the germination phase, informs us about their ability to extend their roots into the soil's deep layers in search of humidity in the event of real drought in the field.

Most of the genotypes whose roots showed an ability to grow despite the stress induced by PEG-6000 at the germination stage (V7; V8; V18; V29; V30) would produce the best yield under drought conditions in the field (V7; V25; V26; V29; V30).



**Fig. 7.** Correlation between barley yield under drought and seedling root length under PEG-6000 stress conditions.



**Fig. 8.** Relationship between grain yield obtained under drought conditions and the root number (RN) obtained at the germination stage in the presence of PEG-600.

This finding is per those of Mishra et al. (2019) and Kim et al. (2020) for which a deep and prolific root system capable of extracting water from the soil would be an essential trait for adaptation to drought. In addition, it is in harmony with those of Maiti (2012), who showed that the root length and its fineness under water stress conditions could be considered reliable criteria to assess the level of drought tolerance of durum wheat. On the contrary, no correlation was found between grain yields obtained in the field under drought conditions, and the root number emitted at the germination stage, in PEG-6000-induced

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stress (Fig. 8). Probably because the dose used was low, enough to induce a substantial reduction compared to the control. Although some authors working on other species have mentioned the reduction of root number (El-Fakhri et al., 2010; Esan et al., 2023) or even an increase in root number under PEGinduced stress (Nupur et al., 2020; Reyes et al., 2023). It is also important to recognize that the stress induced by the PEG does not entirely reproduce the drought conditions in the field, which are very complex and involve the soil nature, the plant species, the variety, and the nutrition conditions. This is why focusing on the effect of PEG-6000 on the root number was not the correct path leading to positive correlations between this parameter (RN) and drought tolerance in the field.

#### 4. Conclusion

This study has allowed us to identify significant positive correlations between Ys and STI on the one hand and between Ys and YSI on the other side. Similarly, significant negative associations between Ys and the indices SSI and TOL showed the effectiveness of (STI and YSI) on the other indices. Among the germination parameters (germination rate, root length, root number, etc.), only root length (RL) was positively and significantly correlated with high yield obtained under drought conditions, in the field. Therefore, it is possible to select the most drought-tolerant genotypes, based on this criterion (RL), at an early stage to assess an important population for its drought tolerance. It would be more interesting, in terms of cost and time saving, to evaluate the drought tolerance of a large number of cultivars, at an early stage, than to evaluate them at a late stage in the field. Moreover, V30 and V7 genotypes were the most productive both under ample and limited water conditions. According to the statistical analysis, these two genotypes were at the top of the ranking and constituted the same cluster. However, V30 showed a slightly higher yield than V7 and presented the highest drought tolerance index; unlike V10 which always exhibited the lowest yield regardless of the water regime used.

**Conflict of interest:** The authors declare that they have no conflict of interests.

**Informed consent:** The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

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