Some Physiological Parameters as Screening Tools for Drought Tolerance in Bread Wheat Lines (Triticum aestivam L.)

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Abstract Two greenhouse experiments were carried out at the Institute of Biological Production Systems, Leibniz Universität Hannover, Germany during 2008/2009 and 2009/2010 growing seasons to study the influence of the osmotic adjustment (OA) capacity, relative water content (RWC) and specific leaf area (SLA) on tolerance to drought in 22 breeding lines, two parents and tolerant cultivar (Sahel 1) of bread wheat (Triticum aestivam L.) under drought conditions. Differences were seen in of the OA, RWC and SLA of the different genotypes. Mean over all of OA, RWC and SLA for breeding lines were -0.51 Mpa, 83.28% and 116.56 cm²g⁻¹, respectively. Four of the breeding lines showed the greatest osmotic adjustment capacities, high RWC and good SLA values under drought stress conditions better than the tolerant cultivar. The heritability of OA, RWC and SLA was 0.56, 0.49 and 0.88, respectively. The results indicated that osmotic adjustment, as well as RWC and SLA could be used as screening tools for drought resistant bread wheat genotypes in the greenhouse. This study also demonstrated the appropriate greenhouse screening methodology in this regard.

Keywords: wheat, drought, osmotic adjustment, heritability

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1. Introduction

Wheat (Triticum aestivum L.) is a staple food for more than 35% of the world population and it is also the first grain crop in Egypt. Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world. In addition to the complexity of drought itself [27,28]. The objective in many breeding programs is to develop cultivars tolerant to drought stress but success has been limited. Genetic improvement of stress tolerance in crop plants requires identification of relevant physiological stress tolerance mechanisms as selection criteria [16] and testing to verify the value of such criteria for improvement of stress tolerance. Osmotic adjustment (OA) is generally considered an important component of drought resistance [15]. Osmotic adjustment (OA) strongly depends on the rate of plant water stress. OA requires time, and fast reduction in plant water status does not allow time for adjustment. This is very significant when genotypes are compared for their OA capacity. However, the importance of the time and the rate of stress for the development of OA imply that OA may not be a very effective mechanism of drought resistance under conditions where the development of drought is by nature very rapid, such on very light tropical or sandy soils of very low water holding capacity [2]. It was recently shown that a population issued from an inland desertic area displayed a higher ability for OA in drought conditions than a population originating from a salt-affected coastal site [24]. These contrasting populations provide interesting material with which to (i) quantify the relative contribution of various osmolytes to OA and (ii) to determine the importance of OA in the adaptative response of Atriplex halimus to water stress.

Leaf relative water content (RWC) was a better indicator of water status than was water potential [18]. Measurements of relative water content (RWC) in leaf tissues are commonly used to assess the water status of plants [37]. Ref. [26] stated that RWC of bean leaves under drought stress significantly was lesser than control. Ref. [14] subjected bean plant to drought stress and after 10, 14 and 18 days after irrigation was with holded, they evaluated RWC of stem and found RWC was significantly lower comparing with control plants. Ref. [9] applied antitranspirant materes on two Sesame cultivars named
Gize 32 and Shanavil 3 and observed that this matters by preventing water transpiration from leaves, led to increase in RWC in these cultivars. Specific leaf area (SLA), an indicator of leaf thickness, has often been observed to be reduced under drought conditions [21].

In the present work the mechanisms involved in the response to drought were investigated in 22 wheat lines with contrasting drought tolerance capacities, in order to study the relationship between the physiological basis of drought response and plant stress tolerance.

2. Material and Methods

The present investigation was carried out in the greenhouse during 2008/2009 and 2009/2010 at the Institute of Biological Production Systems, Leibniz Universität Hannover, Germany. The breeding materials used in this study consisted of all F5 families selected in F4 on the basis of high yield under drought conditions, as well as parents and the tolerant cultivar (Sahel 1). The total number of evaluated families was 22 families. The used genetic material:

| Parental name | Pedigree | Origin |
|---------------|----------|--------|
| Sids 4 (P1)   | May'S/Mon'S//'CMH74A.592/3/Giza 157*2 | Egypt |
| Tokwie (P2)   | ------ | South Africa |
| Sahel 1       | NS 732/PIMA//Veery'S' ICARDA | ICARDA |

Laboratory procedures:

Two experiments were carried out during 2007/2008 and 2008/2009 seasons. Polyvinyl chloride columns of 12.5 cm inner diameter were used and the length of the columns was 50 cm in the two experiments. The substrate for growing the plants was homogenized loamy soil, which was dried in the greenhouse for one month before starting the experiments. While filling the columns, soil samples were taken to determine the initial soil water content. Soil water holding capacity (SWHC) was measured by subtracting the weight of columns filled with dry soil and the weight of columns saturated with water after allowing the exceeding water to drain until there was no change in the weight.

The experiment was designed as RCBD with four replicates in north-south direction, and genotypes were completely randomized within the plots. Two seeds were sown in the middle of every column. After germination, one seedling was removed. Plants were kept well watered at 80% water holding capacity (WHC), Columns were weighed and plants were toppled up every third day to reach required SWHC and the drought stress cycle was started four weeks after sowing and the duration of the stress cycle was about 4 weeks. Fertilizer was applied to the optimum dosage and diseases and pests were controlled using appropriate pesticides.

The following measurements were recorded:

- Relative leaf water content (%) (RWC): was measured at the beginning of the stress cycle and at the end of stress cycle on leaf cuttings, a small part of leaf area of the main tiller and the first biggest other tiller were cut and RWC was calculated using the following equation:

\[
\text{RWC} = \frac{(FW - DW)}{(TW - DW)} \times 100
\]

where FW and DW are fresh weight and dry weight of the leaf and TW is the turgor weight of the leaf after submergence of leaf samples in distilled water for 24 h.

- Osmotic potential (\(\Psi_s\)) was measured at the beginning of the stress cycle and after the stress cycle on leaf cuttings, a small part of leaf area of the main tiller and the first biggest other tiller were cut and stored to measure osmotic potential by using the psychrometric method and Wescor C-52 sample chambers (Wescor Inc., Logan, USA). \(\Psi_s\) was corrected for relative leaf water content. Osmotic adjustment (Mpa), OA was estimated as follows:

\[
OA = \Psi_{scd} \times RWC_{cd} \times \Psi_{sw} \times RWC_{sw}
\]

where \(\Psi_{scd}\) and RWC_{cd} are \(\Psi_s\) and RWC under drought stress conditions, and \(\Psi_{sw}\) and RWC_{sw} are \(\Psi_s\) and RWC under well watered conditions.

3. Results and Discussion

The analysis of variance (Table 3) revealed highly significant differences among the families selected of population I on independent culling levels basis in all physiological traits, i.e., relative leaf water content under well-watered (RWC_{sw}) and relative water content under drought stress conditions (RWC_{cd}), specific leaf area under drought stress (SLA_{cd}) and osmotic adjustment (OA) in the two years.

| Source of variance | D.F | M. S | E. M. S |
|-------------------|-----|------|--------|
| Replication       | r−1 | M1   | \(\sigma^2e\) + \(\sigma^2t\) |
| Genotypes         | g−1 | M2   | \(\sigma^2g\) + \(\sigma^2r\) |
| Error             | (r−1)(g−1) | M1   | \(\sigma^2e\) |

6. Mean comparisons were calculated by using revised L.S.D where, L.S.D = least significant difference, and was calculated as:

\[
R \ L \ S \ D_r = (tα) \sqrt{2MSE/r}
\]

(El Rawi and Khalafalla 1980)

Where \(t\) is the \(t\) value from "minimum-average-risk table" at \(F\)-value of treatments, treatment df and experimental error df.

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![Table 1. The pedigree and origin of the two parents and the tolerant cultivar (Sahel 1)](image)

![Table 2. The analysis of variance and expected means of squares](image)
The combined analysis over two years (Table 4) revealed highly significant differences among families and years for the above-mentioned traits in the two populations. While, families × years interaction was highly significant for SLAcd and OA and non-significant for RWCww and RWCcd in the two populations.

The range and the mean values of the four studied traits within and across the two years are presented in Table 5 and Figure 1. The results showed that average of RWCww was 88.23% with a range from 84.35 to 91.99% in the first season and in the second season, the average was 85.82% with a range from 82.56 to 89.10%. However, mean across years of RWCww was 87.03% with a range from 83.46 to 90.54%. Moreover, the results showed that six families, i.e., no. 6, 13, 22, 23, 24, 25, 26, 28, 33, 38, 45 and 46 in the second season were significantly higher than the best parent. While, twelve families, i.e., no. 6, 19, 22, 23, 24, 25, 26, 28, 33, 38, 45 and 46 in the first season and all breeding lines except (no. 13, 37, 39 and 43) in the second season surpassed the check (Table 7).

Mean SLAcd presented in Table 5 and Figure 3 was 125.77 cm²g⁻¹ with a range from 81.33 to 165.32 m²kg⁻¹ and from 85.15 to 144.58 cm²g⁻¹ with an average 107.36 in the first and second seasons, respectively. However, mean across years of SLAcd was 116.56 m²kg⁻¹ with a range from 86.51 to 154.95 cm²g⁻¹. Twelve families, i.e., no. 6, 19, 22, 23, 24, 25, 26, 28, 33, 38, 45 and 46 in the two years were significantly higher than the best parent.
The average of osmotic adjustment (OA) was −0.56 Mpa with a range from −0.36 to −0.79 Mpa and from −0.23 to −0.68 Mpa with an average −0.47 Mpa in the first and second seasons, respectively. Moreover, mean over all was −0.51 Mpa with a range from −0.37 to −0.68 Mpa (Table 5 and Figure 4). Moreover, five families (no. 22, 25, 26, 33 and 48) and three families (no. 22, 33 and 38) significantly exceeded the best parent in the first and the second seasons, respectively. While, five families, i.e., no. 22, 25, 26, 33 and 48 and four families, i.e., no. 19, 22, 33 and 38 surpassed the best parent in the first and the second seasons, respectively (Table 7). The wide range of variability among genotypes also indicates the suitability of OA as selection tool for breeders under water deficit environments. These results were in agreement with these obtained by [3,16,19,20,22,23]. Also, Ref. [26] showed that Tullio, the drought susceptible Italian genotype, had an osmotic adjustment of −0.63 Mpa, four times greater than in Pandas, though this drought resistant cultivar showed a similar content in osmotically active substances.

Table 7. The average of specific leaf area under drought conditions (SLAcd) and osmotic adjustment (OA) across the two years

| Selected families | Year 1 | SLAcd Year 2 | Mean over all | Year 1 | OA Year 2 | Mean over all |
|-------------------|--------|-------------|--------------|--------|-----------|--------------|
| 1                 | 153.83 | 121.38      | 137.60       | -0.40  | -0.33     | -0.37        |
| 6                 | 99.03  | 85.15       | 92.09        | -0.59  | -0.40     | -0.50        |
| 13                | 148.85 | 127.16      | 138.00       | -0.55  | -0.51     | -0.53        |
| 19                | 127.48 | 112.22      | 119.85       | -0.36  | -0.59     | -0.48        |
| 22                | 116.72 | 97.46       | 107.09       | -0.67  | -0.68     | -0.68        |
| 23                | 119.25 | 102.41      | 110.83       | -0.53  | -0.31     | -0.42        |
| 24                | 81.33  | 91.69       | 86.51        | -0.61  | -0.54     | -0.58        |
| 25                | 101.81 | 87.51       | 94.66        | -0.79  | -0.49     | -0.64        |
| 26                | 123.78 | 117.98      | 120.88       | -0.65  | -0.57     | -0.61        |
| 28                | 88.77  | 90.89       | 89.83        | -0.51  | -0.31     | -0.41        |
| 33                | 106.52 | 85.95       | 96.22        | -0.72  | -0.63     | -0.67        |
| 36                | 135.43 | 100.62      | 118.02       | -0.51  | -0.45     | -0.48        |
| 37                | 142.71 | 131.76      | 137.24       | -0.58  | -0.50     | -0.54        |
| 38                | 115.66 | 99.70       | 107.68       | -0.57  | -0.61     | -0.59        |
| 39                | 165.32 | 144.58      | 154.95       | -0.54  | -0.23     | -0.38        |
| 42                | 146.30 | 118.55      | 132.43       | -0.59  | -0.44     | -0.51        |
| 43                | 148.89 | 135.83      | 142.36       | -0.38  | -0.53     | -0.45        |
| 45                | 106.34 | 88.08       | 97.21        | -0.52  | -0.50     | -0.51        |
| 46                | 118.59 | 97.28       | 108.11       | -0.43  | -0.46     | -0.45        |
| 48                | 133.08 | 96.13       | 114.60       | -0.68  | -0.39     | -0.53        |
| 55                | 132.11 | 108.96      | 120.53       | -0.56  | -0.33     | -0.45        |
| 62                | 154.68 | 120.70      | 137.69       | -0.51  | -0.54     | -0.52        |
| Average            | 125.77 | 107.36      | 116.56       | -0.56  | -0.47     | -0.51        |
| P1                 | 149.60 | 119.70      | 134.70       | -0.44  | -0.48     | -0.46        |
| P2                 | 126.00 | 108.70      | 117.30       | -0.53  | -0.43     | -0.48        |
| Sahel 1            | 126.00 | 114.50      | 120.30       | -0.54  | -0.46     | -0.50        |
| RLSD90             | 4.47   | 9.48        | --           | 0.11   | 0.13      | --           |
| RLSD95             | 9.78   | 12.47       | 0.14         | 0.17   | 0.17      | --           |

The phenotypic (P.C.V.%) and genotypic (G.C.V.%) coefficients of variation and heritability (h²) estimates for all studied traits in the first (2007) and the second (2008) seasons are presented in Table (8). The results showed that the phenotypic and genotypic coefficient of variation were (2.68 and 1.83 %) and (2.52 and 1.37 %) for RWCww, (2.82 and 1.98 %) and (2.87 and 2.00 %) for RWCcd, (17.79 and 17.13 %) and (16.62 and 15.19 %) for SLAcd, and (25.26 and 19.62 %) and (27.20 and 19.85 %) for OA in the first and second seasons, respectively.

Table 8. Phenotypic coefficient of variation (PCV%), genotypic coefficient of variation (GCV%) and broad sense heritabilities (h²) for all studied traits

| Traits | Year  | PCV% | GCV% | $h^2$ |
|--------|-------|------|------|-------|
| RWCww  | 2007  | 2.68 | 1.83 | 46.68 |
|        | 2008  | 2.52 | 1.37 | 29.39 |
| RWCcd  | 2007  | 2.82 | 1.98 | 49.58 |
|        | 2008  | 2.87 | 2.00 | 48.39 |
| SLAcd  | 2007  | 17.79| 17.13| 92.72 |
|        | 2008  | 16.62| 15.19| 83.52 |
| OA     | 2007  | 25.62| 19.62| 58.62 |
|        | 2008  | 27.20| 19.85| 53.25 |

The broad sense heritabilities (Table 8) were (46.68 and 29.39%) for RWCww, (49.58 and 48.39 %) for RWCcd, (92.72 and 83.52 %) for SLAcd and (58.62 and 53.25 %) for OA in the first and second seasons, respectively. The high heritability estimates obtained for both SLAcd and
OA provide evidence for the effectiveness of selection for both characters in improving drought tolerance. These results are in accordance with those obtained by [5,10,11,34,35].

4. Conclusions

Survival and productivity of crop plants exposed to environmental stresses are dependent on their ability to develop adaptive mechanisms to avoid tolerate stress [1]. This study was following to find characters of resistant under drought stress and the results showed that osmotic adjustment, Relative water content and Specific leaf area made difference between genotypes. Thus, this attributes can be used as screening tool for drought tolerance in Wheat. They lend full support to results presented by [16,20,22] showing that wheat lines can differ consistently for OA. It is therefore concluded that OA can be an important component of drought resistance in wheat within a relevant environmental context and by [30] showed that wheat cultivars having high RWC are more resistant against drought stress.

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