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Drought Tolerance Screening and Estimation of Genetic Variation among Some Wheat Cultivars Using Physiological and SRAP markers

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ABSTRACT

The present study was carried out to screen 12 Sudanese wheat (*Triticum aestivum* L.) cultivars for their *in vivo* response to drought stress and to identify diverse sources that could accelerate the development of improved wheat varieties better adapted to dry climate in Sudan. Genetic diversity was studied by sequence-related amplified polymorphism (SRAP) markers. Results of the *in vivo* screening showed significant varietal differences ($p<0.05$) in most of the traits examined indicating the possibility of selection for drought tolerant among the cultivars. Under stress conditions significant ($p<0.05$) reductions in plant height and number of leaves were observed as compared to the control. Significant reduction in stem diameter, head length and head excursion length was only observed when the plants were watered at 7-days intervals. The most susceptible cultivars, that scored the highest Integrated Susceptibility Index (ISI) values, were Imam, Argeen and Sasareeb. Genetic polymorphism indicated that only 24 out of the 64 (37.5 %) markers tested produced different-sized fragments with DNA of one or more of the tested cultivars. The number of fragments detected for each cultivar ranged from 44 for cultivar Imam to 57 for Nebta. The lowest genetic similarity of 55% was detected between cultivars Imam and Tagana, while the highest similarity (86%) was detected between cultivars Condor and Naser. The data generated from morphological, physiological and molecular markers were combined and utilized to construct a similarity matrix based on Jaccard's coefficient. Values indicated that the closest cultivars were Debiera and Condor, while the most distant ones were Tagana and Imam. Principal coordinate analysis (PCoA) showed a scattered pattern for the 12 cultivars with cultivar Imam, which showed the highest morphological mean performance and the highest ISI values, standing far away from all other cultivars. Results of this study will help in the selection of germplasms in wheat improvement breeding programs.

Indexing terms/Keywords

Keywords : Integrated Selection Index; SRAP markers; Wheat cultivars; Sudan.

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1.INTRODUCTION

Wheat (*Triticum aestivum* L.) is the first important and strategic cereal crop for the majority of world's populations. It is one of the most important crops for meeting the world's basic food needs, followed by rice and other grains [1]. Wheat is becoming increasingly popular in Africa, with output up by two-thirds in the past 20 years. Africa is also a heavy wheat importer, especially in urban areas as bread becomes more important [2,3].

Before 1960, apart from small areas in Darfur and Kordofan, Sudan grew wheat only in the northern sector of the Nile valley, and even there only in a limited scale [4]. Then Sudan expanded wheat cultivation in the last decades to latitudes lower than 15°N as a winter crop, occupying the largest area in Sudanese irrigated schemes. Like all other sub-Saharan African countries, average wheat yields in Sudan are very low due to hot and dry climate [5]. In the northern region wheat production constraints are scarcity of both land and water, as wheat is in direct competition with other valuable winter crops such as beans and vegetables [6].

Upon exposure to drought, plants undergo a variety of changes from physiological adaptation to gene expression [7]. Many advances in the understanding of the molecular mechanism of anti-drought and corresponding molecular breeding have taken place [8,9]. Narrow genetic diversity is a problem in breeding for adaptation to biotic and abiotic stresses because there may not be sufficient variation to breed for greater yield under these stressful conditions [10]. Therefore, it will be necessary to investigate the genetic diversity in wheat germplasm to understand and in the future broaden the genetic variation available for breeding [11]. Morphological traits can be used for assessing genetic diversity but are often influenced by the environment. For effective conservation and use of genetic resources, evaluation of genetic variation within collections could be dramatically enhanced by using DNA molecular markers. These DNA markers, when closely linked to genes of interest can be used to select for the desirable allele/s in a marker assisted breeding program [9]. In recent years, many polymerase chain reaction (PCR) based molecular markers have been extensively applied to estimate genetic variability among wheat cultivars [12,13]. SRAP markers have been used successfully in a wide range of plant species such as Medicago sativa [14], Buchloe dactyloides [15], Gossypium [16], Cucurbita [17], Paeonia suffruticosa [18] and *Triticum spp*. [19,20].

Therefore, the objective of this study was to screen for drought tolerance in some local wheat cultivars and to evaluate their genetic diversity using SRAP markers.

2. MATERIALS AND METHODS

2.1 Plant materials:

Seeds of 12 wheat cultivars, representing the commercial elite cultivars grown throughout Sudan, were obtained from Agricultural Research Corporation (ARC), Ministry of Agriculture, Sudan. These were: Tagana, Nebta, Wadi El Niel, Sasareeb, Argeen, Debiera, Niser, Elnielain, Imam, Condor, Bohain and Khalifa.

2.2 Effect of water stress on cultivars' performance:

The effect of water stress on the agronomical traits of each wheat cultivar was assessed under greenhouse conditions. Seeds were sown in pots and watered daily for 15 days or until uniform seedlings have been obtained. Pots were then divided into four sets, the first set was irrigated daily (the control), the remaining three sets were irrigated at either three, five or seven days intervals for 90 days. The experiment was conducted in a Randomized Complete Block Design (RCBD) with three replicates. Agronomical parameters such as Plant height (PH), Head excursion (HE), Head length (HL), Number of leaves (L), Number of reproductive tillers (RT), % leaves yellowing (Y%) and Stem diameter (STD) were then measured and recorded for each experimental set. Relative Water Content (RWC) was determined according to following equation as suggested by [21]:

$$RWC\% = [(FW - DW) / (DW)] \times 100\%$$

where: FW and DW are fresh and dry weight of wheat plants harvested after 90 days of growth.

2.3 Stress susceptibility indices:

Stress Susceptibility Index (SSI) for each cultivar was calculated according to [22] using the following equation:

$$\Delta q = \frac{(Vp - Vs)}{Vp} \times 100\%$$

where; Δq = Reduction % in trait value due to water stress, Vp = value of the trait for the cultivar under control conditions, Vs = value of the trait for the cultivar under stress conditions.

Then, the phenotypic correlations (b) among all traits under water stress conditions were determined and a path coefficient analysis was done to determine the integrated stress susceptibility indices (ISI) as follows:

$$ISI = b_1MP_{i1} + b_2MP_{i2} + \ldots + b_jMP_{ij}$$

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where; MPij= the mean productivity of trait j on cultivar i, bj the weight value of trait j and bj was populated from the average contribution to factor i [22].

2.4 Markers analysis

A total of 64 Sequence Related Amplification Polymorphism (SRAP) primers' combinations [23] were used. The marker assays were conducted following the procedure of [24]. A 25 µl total volume/reaction was used, consisting of 75 ng genomic DNA, 100 ng primer pair, 125 µM dNTPs, 50 mM KCl and 10 mM Tris-HCl, 25 mM $MgCl_2$ and 1 unit Taq polymerase. The amplification procedure consisted of one cycle at 94°C for 3 min, followed by 35 cycles of 1 min at 94°C, 1 min at 47°C, 1 min at 72°C, and a final extension step at 72°C for 5 min. The reaction was then cooled to a resting temperature of 4°C and resolved by electrophoresis in a 12% non-denatured polyacrylamide gel (37:1 acrylamide:bis-acrylamide). The gel was stained in 1 µg/ml ethidium bromide for 10 min, destained in deionized water for 15 min, and photographed using the Gel Doc2000 (Bio-Rad, Hercules, CA.).

2.5 Data analysis:

Analysis of variance (ANOVA) followed by Duncan's Multiple Range Test was used to analyze the morphological and physiological data using SPSS v. 16.0 software package. The genetic similarity between the cultivars was estimated using Jaccard's similarity coefficients (PAST 3.01 software). Morphological, physiological and molecular data were combined and analyzed by Principal Coordinate analysis (PCoA) for cultivars' discrimination.

3. RESULTS AND DISCUSSION:

In order to evaluate their drought tolerance, morphological data were determined for the 12 wheat cultivars grown under water stress conditions as well as under normal watering (control) conditions. Analysis of variance (ANOVA) of the data showed that the plant height, number of spikes, number of leaves, leaves' yellowing %, relative water content (%), stem diameter, head length and head excursion were affected by irrigation regime and cultivar treatments (Table 1). With the exception of yellowing %, significant ($p<0.05$) varietal differences were observed in all traits examined, indicating genetic variation among cultivars and the possibility of selection for drought tolerant among them. Significant variations ($p<0.05$) due to water stress has also been detected between cultivars in all studied traits, the exceptions were the number of spikes and RWC%. However, significant variation due to the interaction between cultivars and stress was only observed in the plant height and No. of spikes. Reduction in plant height due to water stress was also reported by [25] who were evaluating drought tolerance of two spring wheat cultivars. Morphological and agronomic traits are important determinants of crop yield and could be used as yield markers in breeding programs aiming at introducing commercial varieties under end seasonal drought stress conditions [26].

Table 1: ANOVA test for morphological and physiological traits of 12 wheat cultivars grown under four water stress conditions

Source of variation	*df*	Mean squares (SE)							
		Plant height	No. of spikes	No. of Leaves	Yellowing %	RWC	Stem diameter	Head length	Head excursion length
Cultivars	11	137.35** (1.374)	17.74** (0.199)	12.53** (0.177)	309.53^{NS} (4.078)	7451.73** (12.479)	3.051** (0.231)	16.01 ^{NS} (0.862)	35.03** (0.819)
Water stress	3	1480.33** (0.794)	1.45 ^{NS} (0.115)	75.45** (0.102)	505.09* (2.354)	555.32 ^{NS} (7.205)	6.55** (0.133)	60.16** (0.498)	35.37* (0.473)
Cultivars x water stress	33	48.52** (2.749)	1.41** (0.398)	0.42 ^{NS} (0.354)	66.42 ^{NS} (8.156)	1245.06 ^{NS} (24.958)	0.93 ^{NS} (0.462)	9.14 ^{NS} (1.724)	8.34 ^{NS} (1.638)

Results in Table 2 indicate that the highest mean values in plant height, RWC%, stem diameter and head length were recorded for cultivars Argeen, Nebta, Imam and Condor, respectively. However, the highest mean values in No. of spikes, No. of leaves, yellowing % and head excursion were recorded for cultivar Sasareeb. The lowest mean values of plant height, No. of leaves and head excursion length were recorded for cultivar Khalifa while those of stem diameter and head length were recorded for Elnielain cultivar. The highest overall mean (of all studied traits) was recorded for Imam, followed by Nebta, Debiera and Naser while the lowest was recorded for Khalifa followed by Tagana, Elnielain and Bohain.

Under in vivo stressed conditions significant ($p<0.05$) reductions in plant height and number of leaves were observed as compared to the control (Table 3). Significant reductions in stem diameter, head length and head excursion length was

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Table 2: Performance of different wheat cultivars as indicated by different morphological measurements

Cultivars	Means performance								
	Plant Height	No. of Spikes	No. of Leaves	Yellowing (%)	RWC	Stem Diameter	Head Length	Head Excursion length	Overall mean
Debiera	*33.12^{bc}	1.90^{cde}	4.92^{bc}	41.92^{ab}	89.00^{ab}	3.19^{a}	9.20^{abc}	1.85^{de}	**23.14**
Argeen	38.05^{a}	1.33^{e}	4.58^{cde}	39.51^{ab}	45.10^{c}	3.08^{ab}	8.82^{abc}	3.98^{bcd}	**18.06**
Khalifa	26.75^{d}	1.53^{de}	2.92^{g}	42.03^{ab}	37.38^{c}	2.31^{c}	8.44^{bc}	0.93^{e}	**15.29**
Tagana	35.56^{ab}	1.78^{de}	4.92^{bc}	50.86^{a}	28.59^{c}	2.38^{bc}	8.49^{bc}	3.51^{bcde}	**17.01**
Bohain	32.00^{bc}	1.83^{de}	4.83^{bcd}	47.42^{a}	58.36^{bc}	2.70^{abc}	7.71^{bc}	2.17^{cde}	**19.63**
Naser	30.65^{cd}	2.75^{b}	3.58^{f}	42.31^{ab}	87.99^{ab}	3.08^{ab}	8.31^{bc}	3.39^{bcde}	**22.76**
Imam	32.05^{bc}	2.50^{bc}	4.33^{de}	41.54^{ab}	100.78^{a}	3.37^{a}	8.32^{bc}	5.35^{ab}	**24.78**
Condor	37.47^{a}	2.17^{bcd}	5.33^{b}	45.87^{a}	55.43^{bc}	3.28^{a}	11.27^{a}	4.19^{bcd}	**20.63**
Nebta	28.88^{cd}	2.08^{cd}	4.17^{e}	43.08^{ab}	101.48^{a}	2.91^{abc}	7.87^{bc}	4.75^{bc}	**24.40**
Sasareeb	29.61^{cd}	5.92^{a}	6.58^{a}	49.07^{a}	65.25^{abc}	2.62^{abc}	9.84^{ab}	7.23^{a}	**22.02**
Wadi El Neil	30.83^{cd}	1.92^{cde}	5.00^{bc}	32.08^{b}	89.00^{ab}	2.76^{abc}	9.25^{abc}	2.41^{cde}	**21.66**
Elnielain	32.61^{bc}	1.54^{de}	3.00^{g}	48.13^{a}	59.03^{bc}	1.58^{d}	6.67^{c}	3.00^{bcde}	**19.45**
Overall mean	**32.30**	**2.27**	**4.51**	**43.65**	**68.12**	**2.77**	**8.68**	**3.56**	**20.73**

* The mean of mean values at different water regimes

Table 3: Effect of different water rgimes on the overall performance of the cultivars as indicated by morphological measurements

Treatments	Means Performance								
	Plant Height	No. of Spikes	No. of Leaves	Yellowing (%)	RWC	Stem Diameter	Head Length	Head Excursion length	Overall mean
Control	*38.58^{a}	2.47^{a}	6.36^{a}	38.58^{b}	63.85^{a}	3.07^{a}	8.56^{a}	4.00^{a}	**20.68**
3 days stress	34.46^{b}	2.28^{a}	4.86^{b}	43.21^{ab}	73.36^{a}	3.13^{a}	9.62^{a}	4.49^{a}	**21.93**
5 days stress	32.75^{b}	2.35^{a}	3.83^{c}	45.71^{a}	67.81^{a}	2.68^{a}	9.65^{a}	3.57^{ab}	**21.04**
7 days stress	23.41^{c}	1.99^{a}	3.00^{d}	47.10^{a}	67.44^{a}	2.21^{b}	6.90^{b}	2.19^{b}	**19.28**
Overall mean	**32.30**	**2.27**	**4.51**	**43.65**	**68.12**	**2.77**	**8.68**	**3.57**	**20.73**

* The mean of mean values of 12 wheat cultivars

only observed when the plants were watered at 7-days intervals. These results are in line with the results of [27] –[28] who reported the negative effect of drought on many agronomical traits including plant height and spikes length. Leaves yellowing % increased when water was held for more than 3 days. Although the 12 cultivars showed a great variation in RWC%, there was no significant difference in this trait at different stress conditions. [29]-[30] proposed that RWC% was more reliable than other physiological traits for the evaluation of the plant's response to water shortage and that stressed plants have lower RWC% than non-stressed ones. Significant differences in leaf RWC% have been previously reported among the tolerant and intolerant wheat [31]-[32]-[33] barely cultivars.

A better understanding of the morphological and physiological basis of changes in water stress resistance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions [34]. Morphological and physiological data were used to calculate the integrated susceptibility indices (ISI) for the 12 wheat cultivars in order to select the most drought tolerant ones (Fig. 1). The most susceptible cultivars were Imam, Argeen and Sasareeb with ISI values of 1369.52, 982.64 and 754.38, respectively, while the most tolerant cultivars were Debiera, Elnielain and Wadi El Neil with ISI values of -1461.09, -1389.91 and -1269.16, respectively.

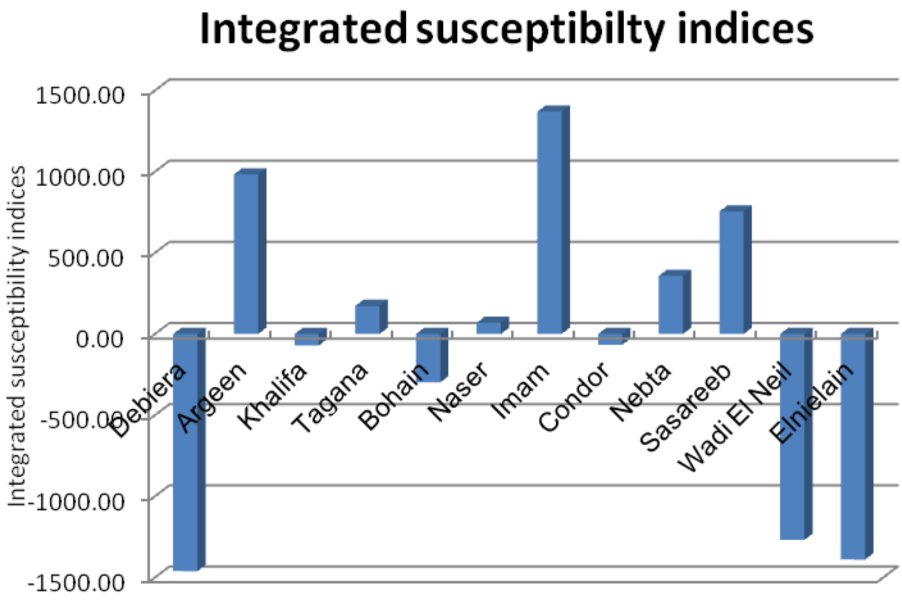

Fig. 1: Response of different cultivars to water stress as indicated by their ISI

Genetic polymorphism of the 12 wheat cultivars was estimated using 64 SRAP markers. Results indicated that only 24 out of the 64 (37.5 %) markers tested produced different-sized fragments with DNA of one or more of the tested cultivars. A total of 681 DNA fragments were detected for the 12 cultivars giving an average of 56.75 alleles per cultivar. The number of fragments detected for each cultivar ranged from 44 (cultivar Imam) to 57 detected for Nebta. Genetic similarity coefficients calculated from SRAP markers' data suggested a relatively high level of genetic diversity among the 12 wheat cultivars (Table 4). The lowest genetic similarity of 55% was detected between cultivars Imam and Tagana, while the highest similarity (86%) was detected between cultivars Condor and Naser as well as between cultivars Khalifa and Argeen. The 12 cultivars were clustered into 5 groups (Fig. 2), in which each of cultivars Bohain, Tagana and Imam were each in a distinct cluster.

The data generated from morphological, physiological and molecular markers were combined and utilized to construct a similarity matrix based on Jaccard's coefficient (Table 5). Values indicated that the closest cultivars, with 86% similarity, were Debiera and Condor, while the most diverse ones were Tagana and Imam for which 62% similarity was detected. Principal coordinate analysis (PCoA) based on Jaccard's similarity matrix showed the scattered pattern for the 12 cultivars, with cultivar Imam, which showed the highest morphological mean performance and the highest ISI values, standing far away from all other cultivars (Fig. 3). The dangers of a narrow genetic base of the world's major domesticated food crops have been well documented [35].

4. CONCLUSION:

The clustering pattern based on molecular and physiological markers have shown considerable potential for estimating genetic diversity among the wheat cultivars. The information obtained in this study will help in the selection of parents and progenies in wheat improvement breeding programs.

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Table 4: Genetic similarity indices based on SRAP markers' data for different wheat cultivars

	Tagana	Nebta	WadiElneil	Sasareeb	Argeen	Debeera	Naser	Elnilain	Imam	Condor	Bohain	Khalifa
Tagana	1.00											
Nebta	0.73	1.00										
Wadi El Neil	0.73	0.78	1.00									
Sasareeb	0.73	0.78	0.81	1.00								
Argeen	0.80	0.77	0.82	0.83	1.00							
Debiera	0.81	0.84	0.83	0.84	0.82	1.00						
Naser	0.73	0.73	0.81	0.76	0.83	0.81	1.00					
Elnielaie	0.77	0.76	0.85	0.76	0.80	0.82	0.77	1.00				
Imam	0.55	0.68	0.71	0.71	0.67	0.62	0.63	0.72	1.00			
Condor	0.83	0.79	0.85	0.79	0.83	0.85	0.86	0.83	0.63	1.00		
Bohain	0.75	0.74	0.74	0.74	0.75	0.74	0.75	0.81	0.61	0.81	1.00	
Khalifa	0.70	0.73	0.84	0.79	0.86	0.75	0.82	0.83	0.71	0.83	0.75	1.00

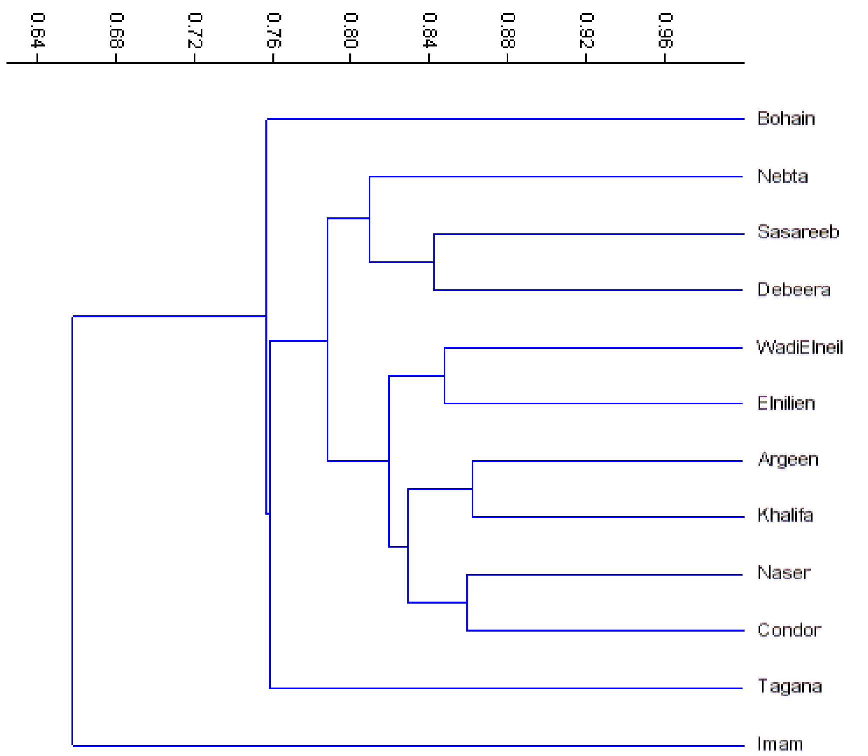

Fig. 2: Dendrogram of 12 wheat cultivars based on data generated from SRAP markers. Values along x-axis correspond to Jaccard's coefficient of similarity

Table 5: Genetic similarity indices based on combined morphological and SRAP markers' data for different wheat cultivars

	Debiera	Argeen	Khalifa	Tagana	Bohain	Naser	Imam	Condor	Nebta	Sasareeb	Wadi El Neil	Elnielain
Debiera	1.00											
Argeen	0.81	1.00										
Khalifa	0.77	0.84	1.00									
Tagana	0.80	0.82	0.71	1.00								
Bohain	0.77	0.74	0.75	0.73	1.00							
Naser	0.78	0.80	0.82	0.75	0.72	1.00						
Imam	0.65	0.74	0.73	0.62	0.63	0.64	1.00					
Condor	0.86	0.81	0.82	0.80	0.82	0.83	0.65	1.00				
Nebta	0.84	0.79	0.76	0.76	0.75	0.74	0.73	0.79	1.00			
Sasareeb	0.84	0.85	0.79	0.77	0.74	0.76	0.76	0.78	0.82	1.00		
Wadi El Neil	0.84	0.83	0.82	0.73	0.75	0.76	0.75	0.84	0.78	0.81	1.00	
Elnielain	0.84	0.78	0.82	0.75	0.84	0.73	0.72	0.83	0.77	0.76	0.84	1.00

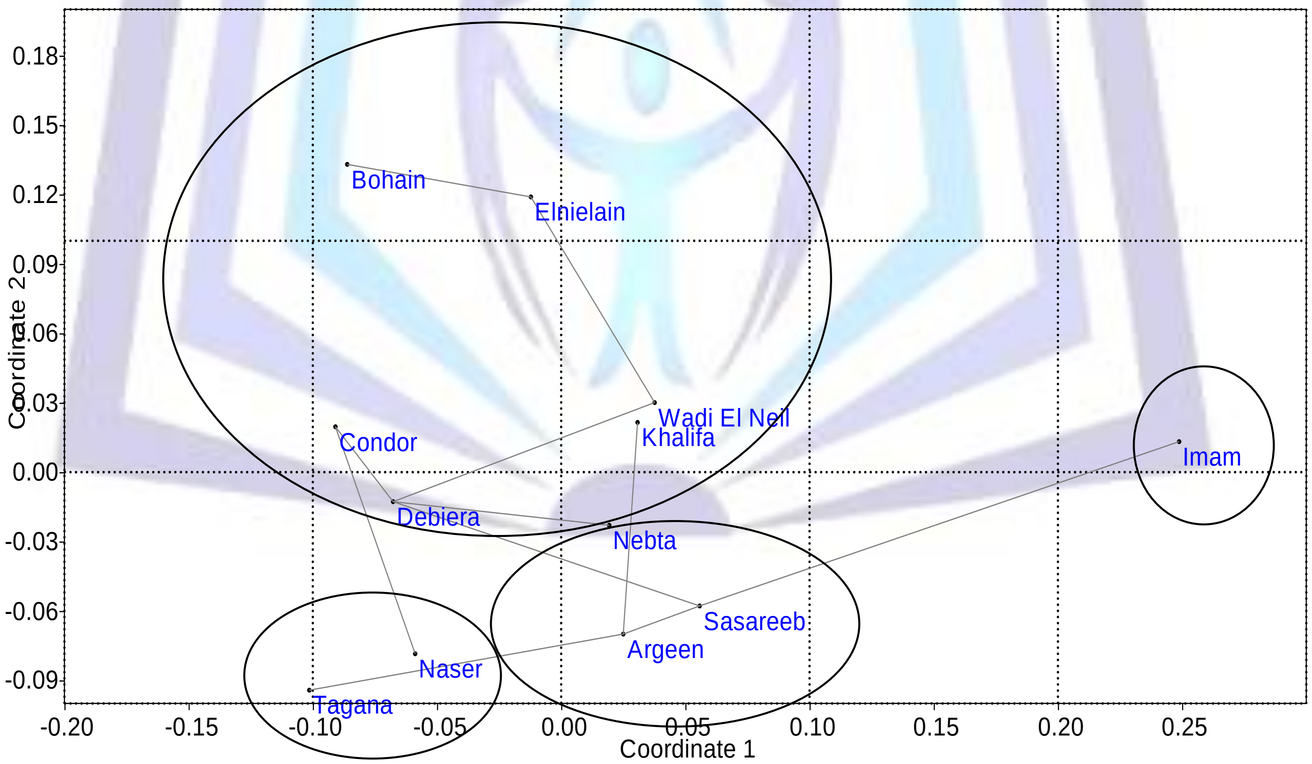

Fig. 3: Two-dimensional view of principle Components Analysis (PCoA) based on the combined data of morphological, physiological and SRAP markers for the 12 wheat cultivars

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