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Stability of Agronomic Traits of Barley Evolutionary Populations under Drought Conditions in Iran

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Abstract: Barley is an important feed crop in Iran and is threatened by an increased frequency of drought. Increasing diversity in the form of evolutionary populations (EPs) and mixtures is one strategy to increase the resilience of crops. Four barley EPs, which have evolved in different locations over 7 to 10 years from the same original population, were evaluated for agronomic trait and stability together with two landraces, and one improved variety for three cropping seasons in four locations. Modest but significant differences were found only for plant height with a range of less than 4 cm. Stability, measured with cultivar superiority, as well as environmental variance and reliability measures generally indicated a superior stability of EPs—with two of them ranking first and second for grain yield reliability—but also differences between the EPs. The effect of recurrent droughts on the diversity within EPs is discussed as a possible explanation for the lack of divergent evolution. The seed management of Eps, including seed exchange between farmers, is suggested as a possible strategy to avoid the reduction in diversity within populations. Future research will address the nutritional value of the EPs, which is often quoted by sheep owners as superior to commonly grown varieties.

Keywords: evolutionary populations; drought; climate change; seed exchange; stability indices; biodiversity

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1. Introduction

There is a consensus among scientists that increasing crop diversity offers a solution to several challenges facing our global food system [1], including climate change [2].

Climate change, both as long- and short-term phenomenon, is increasingly threatening farmers' livelihoods and the in-situ conservation of genetic resources. The loss of agrobiodiversity is associated with an increased vulnerability both to climate change [3], including climate extremes [4], and to agricultural pests [5,6]. This situation is growing even more alarming because of the complexity of climate change, which involves not only a change in temperature and rainfall, but also in the type and spread of insects, diseases and weeds [7].

Iran is particularly vulnerable to climate change because about 33.5% of its agricultural land (6.2 million hectares) is rainfed, and 76% of the rainfed area receives less than 400 mm of annual rainfall [8]. Iran is expected to experience an increase of 2.6 °C in mean temperatures and a 35% decline in precipitation in the next several decades [9] and there is evidence of a downward trend in precipitation in recent decades [10–12]; therefore, there is a need to increase the resilience of crops to reduce the effect of the frequent intensive dry episodes [13].

Barley is the second crop after wheat, both in terms of area (1.6 million hectares) and production (3.4 million tons) [12], because of its resistance to abiotic stresses and its widespread use in livestock feeding. Barley is mostly grown under rainfed conditions with an average yield of 1.1 tons ha^{-1}, and since the total production could not satisfy the internal demand, about 2.5 million tons of barley are imported yearly [12].

Although there are two distinct barley breeding programs in the country, one for irrigated and the other for rainfed areas, the lack of decentralized selection fails to consider the effect of the genotype by location interaction [14] in a country with diverse microclimates in the rainfed areas, resulting in a limited varietal diversity. This is one of the most important challenges of rainfed agriculture and the main cause of the low yield in these areas [15]. Currently, there is an average yield gap of 63% between the actual and the potential yield of rainfed barley in Iran [16], and research on the impact of climate change on rainfed barley production anticipates a decrease in the productivity of this crop in the future due to drought [17]. Therefore, increasing genetic diversity and breeding for specific adaptation would be a low-cost and dynamic strategy for rainfed barley systems, thus increasing productivity and yield in these areas [18].

Agricultural biodiversity can be increased by either increasing the portfolio of cultivated crops, thus reversing the current path toward narrowing agricultural crop production [1], or increasing the number of available varieties through decentralized participatory plant breeding [19]. The Centre for Sustainable Development and Environment (CENESTA) used this strategy by implementing a participatory barley breeding program that allowed the identification of a number of lines superior to the widely grown improved variety Sararood-1 [20]. An additional and complementary strategy is the use of heterogeneous varieties such as evolutionary populations (EPs) and/or mixtures [21,22].

There is a large body of research on EPs (also called bulk populations or composite crosses) demonstrating their ability to adjust their phenology to the environment where they evolve, to become higher yielding, to present a more stable yield and to have a superior resistance to diseases [22], although there are also reports showing that mixtures might not have a yield advantage over the components [23] or a better disease resistance [24]. The evolution of EPs in different locations did not always result in significant yield differences as it is mostly driven by traits associated with local adaptations [22].

In Iran, participatory plant breeding evolved into evolutionary plant breeding in 2008 [25,26] with both bread wheat and barley, but while the EPs were widely adopted by farmers, their merit was never assessed experimentally. Barley is particularly threatened by climate change because it is mostly grown as a feed crop in rainfed areas; and therefore, EPs, which are expected to have a higher stability than uniform varieties, may offer a solution for the traits of interest to growers.

Stability can be defined as static or dynamic [27]. A stable variety (or population, or mixture) for a given trait in the static sense is a variety that, for that trait, remains unchanged across different environments (years and locations). Static stability is analogous to the biological concept of homeostasis [28]: a stable genotype tends to maintain a constant yield across environments. The term "environmental sensitivity" has also been used in this respect, where a greater sensitivity corresponds to a lower stability [29,30]. A stable variety (or population, or mixture) for a given trait in the dynamic sense is a variety that, for a given trait, has an expression in each environment that is always parallel to the mean response of the varieties in the experiment, or, in other words, that has a zero genotype–environment interaction.

Given the increasing frequency of drought events in Iran and the availability in farmers' fields of a number of EPs of an important crop such a s barley, we could test the hypothesis that EPs do provide a more reliable and stable yield in the rainfed areas of Iran.

In this paper, we present the results of three years' evaluation of different EPs cultivated by farmers but derived from the same original population with the objective of generating information on their performance, including their stability, and to understand the reasons for their acceptability by the farmers' community.

2. Materials and Methods

The material used in the trials includes four EPs (entries 1 to 4), two landraces (the local barley of Kermanshah and Shanee barley of Fars) and one improved variety (Sararood-1) (Table 1). The original EP, Sararood-1 and the two landraces are genetically unrelated.

Table 1. The barley material used in the study.

Entry	Name	Name of Farmer	Origin	Abbreviation
1	Barley 1600 Partoee	Naser Partoee	Kermanshah, Ravansar	Partoee_EP
2	Barley 1600 Mahmoudi	Shahriar Mahmudi	Kermanshah, Kerend-e Gharb	Mahmoudi_EP
3	Barley 1600 Kashkooli	Babak Kashkooli	Fars, Firuzabad	Kashkooli_EP
4	Barley 1600 Ardeshiri	Omid Ardeshiri	Khuzestan, Masjed Soleyman	Ardeshiri_EP
5	Sararood-1	Improved	Kermanshah	Sararood
6	Local barley of Kermanshah	Landrace	Kermanshah	Local
7	Shanee barley of Fars	Landrace	Fars	Shanee

The four barley EPs were derived from one initial EP established in 2008 at the International Centre for Agricultural Research in Dry Areas (ICARDA), then in Aleppo, Syria, by mixing an equal number of seeds of 1578 F_2 of barley. The F_2 was derived from a wide array of crosses, from parents ranging from the wild progenitor of cultivated barley, *Hordeum vulgare* subsp. *spontaneum* (C. Koch), to landraces from different countries and to modern varieties, including both two-row and six-row types, to allow the population to adapt to different conditions. The seed of the original population was sent to CENESTA in the autumn of 2008 and given to one farmer, Mr. Shahriar Mahmoudi, hence the name of one of the four EPs. This farmer grew and multiplied the population in 2008 in Kerend Gharb (Kermanshah province) and exchanged the seed with farmers and nomads in other regions in the following years. In 2010, the population was sown in Mr. Naser Partoee's farm in Ravansar (Kermanshah Province). In 2011, Mr. Mahmoudi distributed some seeds to Mr. Babak Kashkooli in Firuzabad (Fars province) and to Mr. Omid Ardeshiri in Masjedsoleyman (Khuzestan province), and they were sown in their fields. Therefore, the four EPs, identified by the names of the farmers (entries 1 to 4), have different numbers of years of evolution in the respective farms. As the farmers used a part of the grain harvested as seeds for the following cropping season without any human selection, the populations evolved in the different locations under the sole effect of natural selection.

Small samples of the four populations were taken from the farmers in 2018 to be used in this experiment. The choice of the two landraces and the improved variety to be used as controls was based on the farmers' widely cultivated varieties in each location. Shanee barley of Fars and the local barley of Kermanshah are landraces widely cultivated by farmers in temperate areas of each province. Sararood-1 is the most commonly grown improved variety in the rainfed cold and temperate regions of Kermanshah and Fars provinces.

The trials were conducted under rainfed conditions for three years (2020 to 2022) in four locations belonging to three different provinces (Table 2). In each location and year, a part of the grain harvested from the four EPs was used to plant the trials in the following year. In Fars province, the province with the largest barley cultivation in the country, two locations were included as they were used to represent a predominantly rainfed (Firuzabad) and a predominantly irrigated (Naghshe Rostam) area. However, because of water shortage, the farmers in Naghshe Rostam are increasingly switching to rainfed cultivation and are in need of genetic material different from the one they were using in the past. The agronomic management (weeding, fertilizer and irrigation) was conducted as per the farmers' practice of the respective growing areas. No inputs were used in the trials except in Ravansar, where a small amount of fertilizer (24 kg ha^{-1} of phosphate fertilizer and 12 kg ha^{-1} of nitrogen fertilizer) was applied after planting.

Table 2. The locations where the trials were conducted.

No.	Province	Location Name	Type of Management
1	Kermanshah	Ravansar, Morad Abad	Rainfed
2	Fars	Naghshe Rostam, Hosein Abad	Rainfed *
3	Fars	Firuzabad, Mahkuye Bala	Rainfed
4	North Khorasan	Farouj	Rainfed

* Previously used to be irrigated.

2.1. Experimental Design and Data Recording

The experimental design was a randomized block with three replications. The plot size was 20 m^2 in all locations and years. A different randomization for each of the four locations and each year was generated by DiGGer, a program that generates efficient experimental designs for non-factorial experiments with plots arranged in rows and columns [31,32].

The following traits were measured or calculated:

1. Grain yield (kg ha^{-1}) as the average of the grain weight harvested in three quadrats of 1 m^2 each;
2. Biomass yield (kg ha^{-1}) as the average of the total biomass weight harvested in three quadrats of 1 m^2 each;
3. Harvest index = (grain yield/total biomass yield) $\times$ 100;
4. 1000 kernel weight (g) as the average of three samples of 250 kernels each x 4;
5. Number of fertile tillers per m^2 in one of the quadrats used to estimate grain and biomass yield;
6. Plant height (cm) from the base of the plant to the bottom of the spike;
7. Spike length awns excluded (cm).

Traits 1 to 5 were measured or calculated on a plot basis, while traits 6 and 7 were measured on 20 plants/plot (10 in location 4 in 2020).

2.2. Climatic Data

The first year of the experiment was the wettest in all four locations (Figure 1) with 457, 382, 408 and 314 mm of total annual rainfall in Ravansar, Naghshe Rostam, Firuzabad and Farouj, respectively. The year 2021 was the driest year in Ravansar and Firuzabad, while Naghshe Rostam was affected by two very dry years in 2021 and 2022 with as little as 156 and 150 mm of rainfall. Maximum temperature reached its highest in 2021 in Ravansar, Firuzabad and Farouj, and in 2022, the driest year, in Naghshe Rostam. In Farouj (North Khorasan), both maximum and minimum temperatures were lower than in the other three locations.

2.3. Statistical Analysis

The data collected on individual plants were submitted to a combined analysis of variance using the ANOVA command for unbalanced design in GenStat 22nd edition [33], using a random model in which the entry trait Y*ijz* was a function of the grand mean (μ) of the entry (*Ei*) effect of the *i* entry; of the location (*Lj*) effect of the *j* location; of the *(EL)ij* interactions effect; of the year-within-location effect (*Y(L)y*); of the *EY(L)iy* interaction effect; of the plants within entries (*PEi*) effect and of the residual *e*:

$$Yijz = \mu + Ei + Lj + Y(L)y + (EL)ij + EY(L)iy + PE_i + eijyz$$

In the case of data collected on a plot basis, we used the ANOVA command for a general analysis of variance in GenStat 22nd edition [33], using the same random model excluding the effect of the plants within entries.

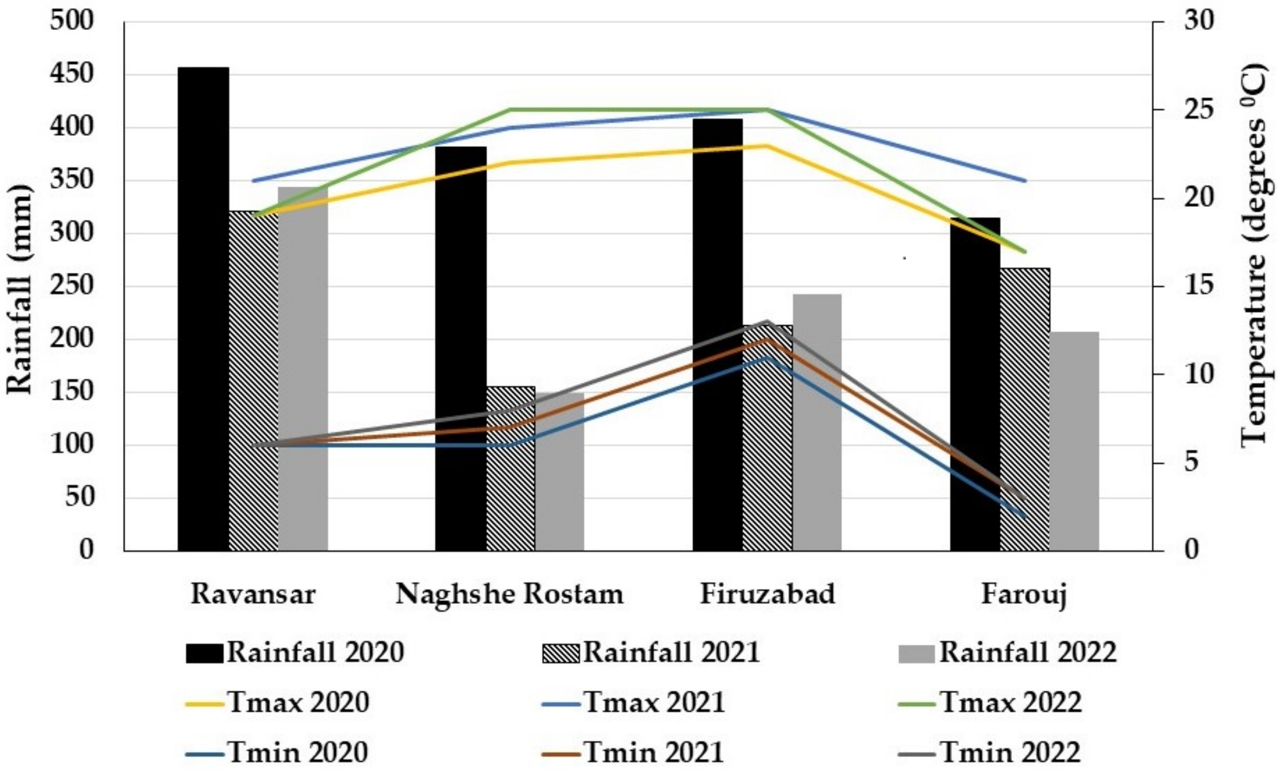

Figure 1. Annual rainfall (mm), maximum and minimum temperatures (degrees °C) at the four locations where the trials were conducted for three years (from Iran Meteorological Organization https://irimo.ir/ accessed on 15 April 2023).

The reasons for adopting this model are given by Singh et al. [34]. When locations are reasonably far from each other (as was the case in these multi-location trials), then the constituents of the 'year' factor vary with location each year. In such a case, the location by year interaction cannot be interpreted; however, the spatial interaction of entry with location, and the temporal interaction of entry with year within locations, can be interpreted. In addition, such a situation does not require the location and year classifications to be connected, because the nesting of years within location is sufficient to allow the estimation of *EL* and of within-location *EY(L)* interactions.

The relationships between mean and stability were analyzed by the GGE biplot package [35] available in R [36]. In the "mean and stability" feature, a line (the mean environment axis) is drawn, which passes through the origin representing the mean of each entry in all years. The projections of each of the entries tested in the experiment to the mean environmental axis approximates the *EY* interaction associated with that entry. The longer the projection, the greater is the *EY* interaction, which is a measure of the instability. In this graphical representation, the ideal entry, in terms of grain yield and stability, is one that has the longest positive projection on the mean environment axis (high mean) and a zero projection on the perpendicular axis and is represented by a circle [35].

To validate the information about the stability of different entries provided by the biplot, we calculated three additional stability indexes, namely the cultivar superiority measure proposed by Linn and Binns [37], the environmental variance [38], and the yield reliability measure proposed by Kataoka [39]. The cultivar superiority measure (CS) of an entry is the sum of the squares of the differences between its yield in each environment and the maximum yield in that environment divided by twice the number of environments. A superior entry is one with a small cultivar superiority measure, meaning that its yield is always close to the maximum [40]. The environmental variance (EV) is a measure of static stability. Entries with a small EV maintain a constant yield across environments. EV has a high repeatability and is independent from the set of tested entries, allowing for a broader generalization [40], The yield reliability measure of an entry j (Ij) combines the mean yield of the entry j (mj) with EVj, as shown in the formula below, where $Z_{(P)}$ is the percentile from the standard normal distribution for which the cumulative distribution function reaches the value P, namely the probability of the lowest yield. We used P = 0.80, namely the lowest yield expected in 80% of the cases corresponding to Z = 0.842, which discriminated between crop failure (negative grain yield expected for some entries) and positive yields:

$$Ij = mj - Z_{(P)} \sqrt{EVj}$$

Cultivar superiority and the environmental variance were calculated using the "GEstability" procedure of GenStat version 20.1 [33] as well as with the R package toolStability [41,42]. The values of various stability indices will be presented together with the ranks of the seven entries (from 1 = most stable to 7 = least stable).

3. Results

3.1. Means of Agronomic Data

There were highly significant effects of both locations and years within locations for all the six traits measured (Table 3). Entries by locations' interactions and entries by years within locations' interactions were significant in the case of 1000 kernel weight, plant height and spike length, while years within locations were significant for all the six traits.

Modest but significant differences between entries were found only for plant height, with the Partoee_EP being the tallest and significantly taller than all the other entries except Ardeshiri_EP (Table 4). With the exception of Kashkooli_EP, the EPs were significantly taller than both Sararood-1, the improved variety, and the local barley from Kermanshah.

Table 3. ANOVA of grain yield (GY), biomass yield (BY), harvest index (HI), 1000 kernel weight (KW), plant height (PH) and spike length (SL) of four barley EPs, one improved variety and two landraces, evaluated in farmers' fields during three years (2020 to 2022) in four locations in Iran.

Source of Variation	GY [a]	BY [a]	HI	KW	PH	SL
Entries (E)	13.1	43.1	0.003	44.6	1223.8 *	29.6
Locations (L)	792.6 ***	2341.0 ***	0.688 ***	1508.6 ***	19,477.7 ***	1058.4 ***
EL	8.3	106.2	0.004	97.9 ***	349.9 ***	18.1 ***
Years within L (Y_L)	2729.9 ***	29,260.0 ***	0.418 ***	1472.1 ***	176,175.3 ***	456.2 ***
EY_L	8.6	97.1	0.006	40.5 **	300.1 ***	7.3 ***
Plants within E	-	-	-	-	44.2	2.4
Residual	11.8	123.5	0.007	21.2	59.3	2.9
Total	26,845.9	273.3	0.027	79.2	405.0	4.5

[a] Original values $\times 10^{-4}$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; plants within entries are included only for the two traits (PH and SL) measured on a plant basis.

Table 4. Means [a] of grain yield (GY), biomass yield (BY), harvest index (HI), 1000 kernel weight (KW), plant height (PH) and spike length (SL) of four barley EPs (entries 1–4), one improved variety (entry 5) and two landraces (entries 6 and 7), evaluated in farmers' fields during three years (2020 to 2022) in four locations in Iran.

Entry	Name	GY (kg ha^{-1})	BY (kg ha^{-1})	HI (%)	KW (g)	PH (cm)	SL (cm)
1	Partoee_EP	874.0	3230	27.9	35.3	52.7 a	6.7
2	Mahmoudi_EP	831.0	3268	26.6	33.9	51.7 b	6.4
3	Kashkooli_EP	879.0	3315	27.2	35.6	49.4 cd	6.4
4	Ardeshiri_EP	725.0	3236	25.3	33.4	51.8 ab	6.7
5	Sararood-1	801.0	3548	25.6	33.2	50.1 c	6.6
6	Local	849.0	3301	27.4	34.9	49.0 d	6.4
7	Shanee	904.0	3273	26.3	32.7	51.6 b	6.9

[a] Means with a letter in common are not significantly different ($p < 0.05$) based on L.S.D. test.

Ravansar, the location with higher rainfall, particularly in the last two years of the experiment (Figure 1), had the highest average harvest index, 1000 kernel weight and plant height. Naghshe Rostam had the significantly highest grain and biomass yield and among the lowest harvest index, plant height and spike length. Firuzabad and Farouj were the locations with the lowest grain yield (Table 5).

Table 5. Means [a] of grain yield (GY), biomass yield (BY), harvest index (HI), 1000 kernel weight (KW), plant height (PH) and spike length (SL) in four locations in Iran of four barley EPs, one improved variety and two landraces, evaluated in farmers' fields in three years (2020 to 2022).

Name	GY (kg ha^{-1})	BY (kg ha^{-1})	HI (%)	KW (g)	PH (cm)	SL (cm)
Ravansar	1007 b	3042 b	39.2 a	41.42 a	55.1 a	6.5 b
Naghshe Rostam	1255 a	4220 a	16.7 c	32.42 b	47.0 c	5.3 c
Firuzabad	509 c	3059 b	19.5 c	31.45 b	46.6 c	6.6 b
Farouj	580 c	2920 b	31.0 b	31.21 b	54.2 b	7.7 a

[a] Means with a letter in common are not significantly different ($p < 0.05$) based on L.S.D. test.

The "mean and stability" feature of the GGE biplots suggest the presence of a wide range of stability levels among the entries (Figure 2A–F).

3.2. Genotype by Environment Interactions

In the case of grain yield (Figure 2A), the most stable entries were two EPs, namely Mahmoudi_EP and Ardeshiri_EP and the landrace Shanee, while the least stable were Kashkooli_EP, the improved variety Sararood-1 and Partoee_EP. The local landrace from Kermanshah (local) was intermediate. It is worth noticing that none of the entries yielded consistently (poor or good) in all the three years in the same location as indicated by the position of the entry labels relative to the environment (combination of location and year) labels. For example, Shanee, the local, Mahmoudi_EP and Sararood-1 yielded more than the average in Ravansar (Rav) in 2021 and 2022, but less than the average in the same location in 2020. Similarly, Kashkooli_EP and Partoee_EP yielded more than the average in Farouj in 2020 and 2021, but less than the average in the same location in 2022.

For biomass yield, two Eps (Partoee_EP and Ardeshir_EP) were the most stable, while the other two EPs, the improved variety Sararood-1 and the landrace Shanee, were very unstable (Figure 2B). The biplot also shows the extent of the variability between years within locations as in no cases the position of a location is consistently on the same side of the mean environmental axis.

Mahmoudi_EP and the local were the most stable for harvest index (Figure 2C), while the landrace Shanee and the improved variety Sararood-1 were the most unstable. The other three EPs had an intermediate level of stability, lower than Mahmoudi_EP and the local landrace, but higher than the landrace Shanee and the improved variety Sararood-1.

Mahmoudi_EP and Shanee and, to a lesser extent, Sararood-1 were very stable for 1000 kernel weight (Figure 2D), while Ardeshiri_EP was most unstable as it had a higher-than-average 1000 KW in Farouj and in Firuzabad in 2021 and 2022, respectively, but a much lower 1000 kernel weight than the mean, particularly in Naghshe Rostam in 2020 and 2021.

All entries were very unstable for plant height with the exception of the local landrace from Kermanshah (Figure 2E). Three EPs (Mahmoudi_EP, Ardeshiri_EP and Kashkooli_EP) were taller than the mean in 2020 in all locations, but shorter than the mean in 2021 and 2022, particularly in Farouj and Naghshe Rostam.

Spike length was very unstable in all entries, mostly as a consequence of variation between years within locations except for Farouj (Figure 2F). In this location, and consistently over the years, spike length was longer than average in the EPs (Mahmoudi_EP, Ardeshir_EP and Partoe_EP) and Shanee, but shorter in Kashkooli_EP, Sararood-1 and the local.

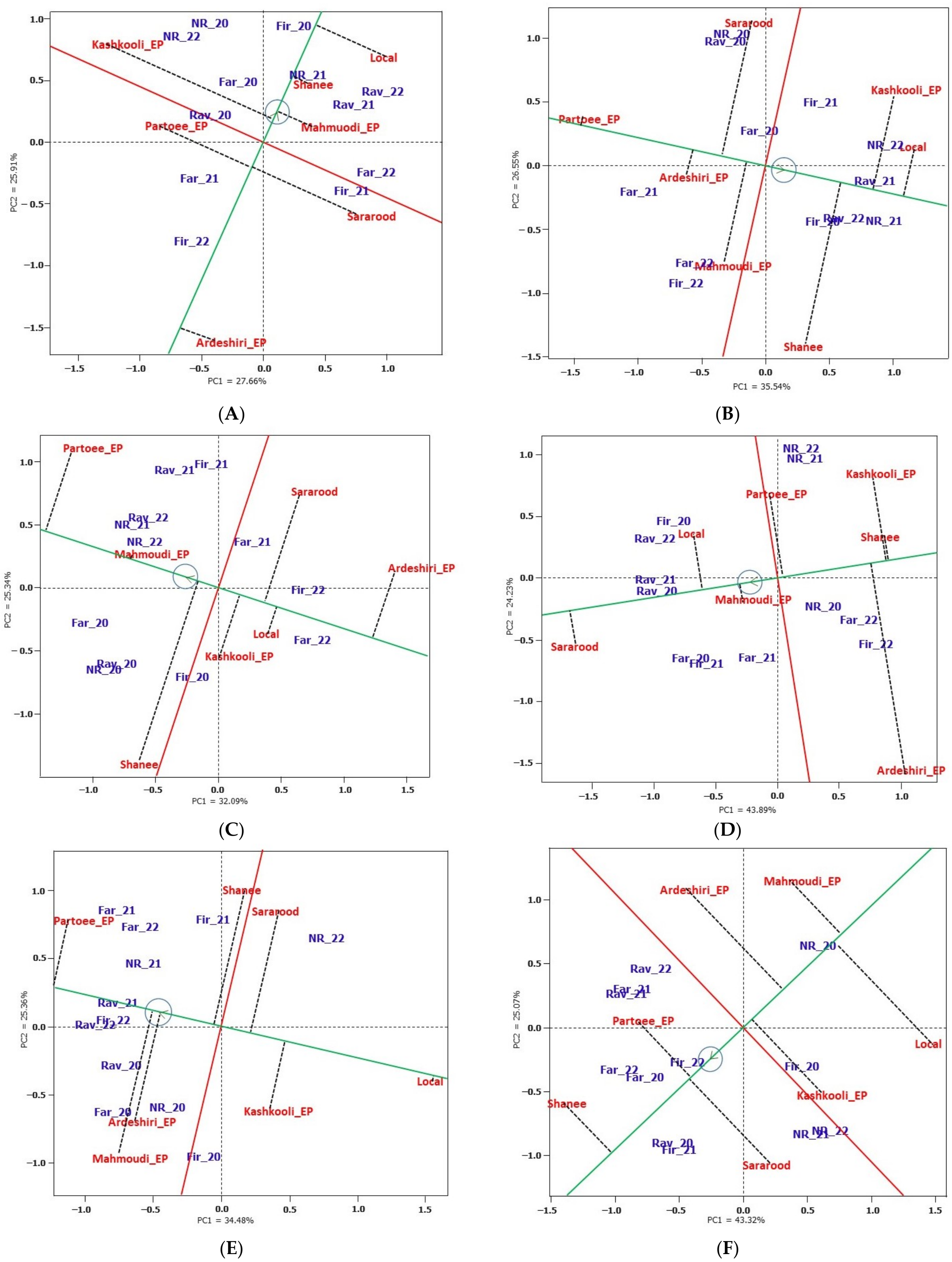

Figure 2. Mean and stability feature of the GGE biplot for grain yield (**A**), biomass yield (**B**), harvest index (**C**), 1000-kernel weight (**D**), plant height (**E**) and spike length (**F**) measured in four barley EPs, one improved variety and two landraces in four locations and three years (Rav = Ravansar; NR = Naghshe Rostam; Fir = Firuzabad; Far = Farouj; 20, 21 and 22 indicate 2020, 2021 and 2022, respectively). The green line is the mean environmental axes, while the red line measures the entries x environment interaction (see material and methods).

3.3. Stability of Agronomic Traits

The indices estimating stability, calculated to validate the information provided by the GGE biplots, are presented in Table 6. There were large differences between EPs for the stability of grain yield regardless of the index. Two EPs (Kashkooli_EP and Partoee_EP), together with Shanee, had the lowest cultivar superiority (i.e., better), while the other two EPs (Mahmoudi_EP and Ardeshiri_EP) were the worst, together with Sararood-1. In terms of static stability (EV), Ardeshiri_EP was the most stable while Partoee_EP was the least stable. Two of the EPs, Ardeshiri_EP and Mahmoudi_EP, were the only two entries that are not expected to suffer from a crop failure in 80% of the cases, as suggested by their positive value of the yield reliability index. Based on this index, the improved variety Sararood-1 is the least reliable entry in terms of grain yield.

Table 6. Stability expressed as cultivar superiority (CS), environmental variance (EV) and index of yield reliability (I) with their relative ranks (1 = most stable to 7 = least stable), calculated for grain yield (GY), biomass yield (BY), harvest index (HI), 1000 kernel weight (KW), plant height (PH) and spike length (SL) measured in four barley EPs (entries 1 to 4), one improved variety (entry 5) and two landraces (entries 6 and 7) evaluated in 12 environments (four locations x three years). Indices followed by the same letter(s) are not significantly different ($p < 0.05$) according to Duncan's test (cultivar superiority) and Ekbohm's [43] variance ratio test (environmental variance).

No.	Entry	GY					
		CS	CS rank	EV	EV rank	I	I rank
1	Partoee_EP	27,566 e	3	1,352,083 a	7	−105.3	6
2	Mahmoudi_EP	35,649 c	5	1,028,482 c	3	19.9	2
3	Kashkooli_EP	20,341 g	1	1,231,444 ab	6	−103.9	5
4	Ardeshiri_EP	102,486 a	7	716,445 d	1	165.7	1
5	Sararood-1	59,357 b	6	978,544 c	2	−108.8	7
6	Local	31,112 d	4	1,106,394 bc	5	−85.0	4
7	Shanee	23,929 f	2	1,102,642 c	4	−36.2	3
		BY					
		CS	CS rank	EV	EV rank	I	I rank
1	Partoee_EP	650,799 a	7	10,853,968 bc	5	455.7	5
2	Mahmoudi_EP	545,709 c	5	9,449,736 cd	2	641.3	3
3	Kashkooli_EP	428,561 f	2	11,275,881 b	6	440.2	6
4	Ardeshiri_EP	510,965 d	4	10,643,109 bc	4	568.2	4
5	Sararood-1	230,115 g	1	13,640,587 a	7	126.6	7
6	Local	479,302 e	3	10,264,389 bc	3	850.3	1
7	Shanee	641,807 b	6	8,915,695 d	1	786.9	2
		HI					
		CS	CS rank	EV	EV rank	I	I rank
1	Partoee_EP	0.002031	3	0.0423 ab	6	0.1467	3
2	Mahmoudi_EP	0.003109	7	0.0392 bc	5	0.1439	4
3	Kashkooli_EP	0.001362	1	0.0371 bcd	3	0.1304	5
4	Ardeshiri_EP	0.002569	4	0.0385 bcd	4	0.1484	1
5	Sararood-1	0.002679	5	0.0481 a	7	0.1123	7
6	Local	0.001687	2	0.0370 cd	2	0.1193	6
7	Shanee	0.002885	7	0.0337 d	1	0.1475	2

Table 6. *Cont.*

		KW					
		CS	**CS rank**	**EV**	**EV rank**	**I**	**I rank**
1	Partoee_EP	9.48 cd	2	69.78 c	3	28.3	2
2	Mahmoudi_EP	23.51 bc	4	80.18 c	5	26.4	5
3	Kashkooli_EP	6.57 d	1	48.41 d	1	29.7	1
4	Ardeshiri_EP	38.00 ab	6	70.48 c	4	26.3	6
5	Sararood-1	45.09 a	7	133.81 a	7	23.5	7
6	Local	19.94 cd	3	101.34 b	6	26.4	4
7	Shanee	24.33 bc	5	50.48 d	2	26.7	3
		PH					
		CS	**CS rank**	**EV**	**EV rank**	**I**	**I rank**
1	Partoee_EP	2.49 c	1	360.46 bc	2	37.2	1
2	Mahmoudi_EP	6.01 bc	3	452.09 a	7	34.4	5
3	Kashkooli_EP	15.93 ab	6	395.12 abc	5	33.6	6
4	Ardeshiri_EP	4.86 bc	2	407.38 ab	6	35.4	3
5	Sararood-1	13.35 abc	5	377.83 bc	3	34.6	4
6	Local	21.04 a	7	378.00 bc	4	33.4	7
7	Shanee	7.99 bc	4	330.86 c	1	36.5	2
		SL					
		CS	**CS rank**	**EV**	**EV rank**	**I**	**I rank**
1	Partoee_EP	0.5734 ab	3	1.725 b	4	5.531	1
2	Mahmoudi_EP	0.7025 ab	5	1.732 b	5	5.282	5
3	Kashkooli_EP	0.8592 ab	6	1.684 bc	2	5.261	7
4	Ardeshiri_EP	0.5539 ab	2	1.810 b	6	5.519	2
5	Sararood-1	0.5881 ab	4	1.709 bc	3	5.507	3
6	Local	0.9146 a	7	1.452 c	1	5.351	4
7	Shanee	0.0807 b	1	4.063 a	7	5.277	6

In the case of biomass yield, an important trait in a feed crop such as barley, Sararood-1 and Kashkooli_EP were the best entries in terms of cultivar superiority, while in terms of environmental variance and yield reliability index, Mahmoudi_EP, the local Kermanshah and Shanee were the best entries—not necessarily in that order. Sararood-1 had the highest environmental variance and the lowest yield reliability index.

While in the case of grain yield, only two entries were able to yield in 80% of the cases, in the case of biomass yield, all the entries were capable of producing some animal feed in 80% of the cases.

There were no significant differences for cultivar superiority in the case of harvest index, while the two landraces were the most stable, having the lowest EV, and Sararrod-1 was the least stable. Not surprisingly, Ardeshiri_EP and Sararood-1, which had the highest and the lowest reliability index for GY, respectively, also had the highest and the lowest reliability index for harvest index.

Kashkooli_EP and Partoee_EP had the best cultivar superiority index for 1000 KW, and Sararood-1 was the worst. Kashkooli_EP was also the most stable in a static sense, having the lowest EV and being able to maintain the highest 1000 kernel weight in 80% of the cases. Partoee_EP ranked as the third best and second best for EV and reliability index, respectively. For this trait, Sararood-1 was consistently the worst entry for the three stability indexes.

Partoee_EP had the most stable plant height when stability was measured as cultivar superiority, followed by Ardeshiri_EP and Mahmoudi_EP. Partoee_EP had the second lowest EV after Shanee, and together with Shanee, had the highest reliability index, i.e., their plants were taller than the other entries in 80% of the cases. In the case of plant height, the local Kermanshah was the worst for cultivar superiority and reliability index.

Kashkooli_EP and Shanee were the most stable entries for spike length when stability was estimated by the cultivar superiority. Kashkooli_EP also had a low environmental variance together with the local Kermanshah. In terms of reliability, i.e., the ability to have a spike longer than the other entries in 80% of the cases, there were no differences between the entries.

4. Discussion

This study was conducted to evaluate four barley EPs, an important feed crop in Iran particularly vulnerable to climate change, being a typical crop of rainfed areas as in most of the Near East [44]. The populations used originated from the same population and had evolved in different locations in Iran for a number of years ranging from 7 to 11. They were also widely adopted by farmers in the provinces where they evolved. It was therefore unexpected to discover that the populations diverged significantly only for plant height, and even for this trait, the differences were quite modest given the length of time they were cultivated in different locations. These results differ from those reported by several studies and summarized by Ceccarelli and Grando [7,22], showing the ability of EPs to become more productive than control varieties, but also to agronomically and morphologically diverge when they evolve in contrasting environments. It is known that directional selection in natural populations of plants and animals is a powerful mechanism to produce microevolutionary changes [45,46] and that directional natural selection is forecasted to increase with more frequent droughts and rising temperatures [47]. The occurrence of severe drought increased during recent years, as reported, for example, by the Iranian Meteorological Organization, which, in April 2021, warned of an "unprecedented drought" and rainfall levels substantially below long-term averages; the amount of rainfall in Iran's main river basins from September 2020 to July 2021 was, in most places, substantially lower compared with the period only a year earlier [48]. Therefore, one possible explanation for the lack of evidence of divergent selection between Eps with a strong directional selection in the same direction could be the recurrent drought that resulted in an increase in the frequency of similar phenotypes in the four populations. This undesirable effect of a strong directional selection could be eventually reduced by two strategies. The first is by avoiding using all the saved seeds for planting, and instead spare some to add to the new harvest; this will at least slow down the decay of diversity within populations. The second is to regularly exchange seeds with neighbors; since there are many paths to drought tolerance [49], this may further reduce, in combination with the first strategy, the progressive reduction in diversity expected with directional selection. On the other hand, the common practice of seed exchange among farmers may cancel out the effect of differences caused by directional selection between EPs grown in different locations.

During the period between the initial sowing of the EPs in 2008 and the beginning of the study, there was a widespread adoption of the Eps, particularly in the Kermanshah province, as this was the first province in which they were planted. The results of this study would suggest that the adoption was not driven by the traits we measured (grain and biomass yield, 1000 kernel weight and spike length), probably not even by plant height (given the modest differences) that is a very important trait in a crop like barley mostly grown as animal feed [14]. The importance of plant height is due to its relationship with the amount of straw and with the possibility of mechanical harvesting, which becomes increasingly problematic when the crop is too short as a consequence of drought. However, the minor, albeit significant, differences in plant height would hardly justify a preference for the EPs.

We may assume that the seed sovereignty that farmers acquired by using EPs [26] could have been an important factor driving adoption even in the absence of a clear agronomic advantage. In fact, in the areas where EPs have been adopted, the use of farmer-saved seeds is a common practice. Another possible driver in adoption could have been advantages in traits not measured in this study, such as disease resistance and weed control. Diseases were not recorded during the trials because the prevalent dry conditions did not allow for their development, and weed control is technically difficult to study in plot experiments because there is usually an uneven distribution of weeds. Furthermore, according to anecdotal evidence, it appears that barley EPs have a superior quality as feed for small ruminants. Barley straw is an important source of small-ruminant feed and its quality is of primary interest [50,51]; moreover, farmers in the Near East place great importance to straw quality in their assessment of new varieties.

The stability of yield, defined as constant yield (static stability), has been considered as important as yield for crop adaptation to poor environments [52], such as those where the majority of barley is grown in Iran. There is evidence in the literature that EPs are more stable than mixtures and pure lines as shown in lima bean [53], in winter wheat [40,54,55] and in barley [56]. It was therefore interesting in this study to discover that the four EPs differed in their stability in some cases regardless of the methods used for estimating it. Moreover, at least one of the EPs was the most stable entry based on at least one of the stability indices, with the only exception of biomass yield. For this trait, there was a disagreement between the information generated by the biplot with Partoee_EP and Ardeshiri_EP as the most stable, while Kashkooli_EP was the second most stable based on cultivar superiority, and Mahmoudi_EP was the second most stable based on environmental stability. This suggests the usefulness of using different methods to assess stability.

5. Conclusions

The objective of this research was to generate information on the agronomic performance and stability of four barley EPs in Iran compared to an improved variety and two landraces widely grown. Comparisons between populations that have evolved through natural selection and those that have been improved demonstrate that, in some cases, the former can perform equally or even better in their native environments. Furthermore, populations that result from a combination of natural and artificial selection have shown both spatial and temporal stability in their performance and stability.

The EPs were derived from the same original population made available to Iranian farmers and nomads in 2008 by CENESTA. The four EPs were grown in four different farms of three provinces for a number of years (from seven to eleven years) prior the trials described in this paper, using farmer-saved seeds, and were widely adopted. The lack of significant differences for the agronomic traits measured during the three years in four locations was somewhat unexpected—the difference in plant height was significant but numerically small—in the light of other published evidence of significant divergent evolutions between barley EPs and EPs of other crops grown in contrasting environments. There was, however, evidence of an overall superior stability particularly in comparison with the improved variety used as control. In discussing the contradiction between the lack of evidence for an agronomic superiority of the EPs and their widespread adoption, we conclude that, in addition to their stability, there might be other factors driving the farmers' preferences. In some cases, and through anecdotal evidence, albeit by nomadic communities, this was either due to the introduction of additional diversity in farms or a superior EP feed quality that could not be measured in these trials and will instead be the specific objective of further studies.

Climate change presents a complex and rapidly evolving problem, as changes in temperature, rainfall and extreme weather events can have unpredictable effects on the interactions between crops and pests, insects, diseases and weeds. Adaptive solutions are required to address this multifaceted issue. One promising solution is the use of evolutionary populations, which possess the ability to adapt to both biotic and abiotic stresses and can do so quickly and cost-effectively, so long as they have sufficient initial genetic diversity. In addition to these benefits, evolutionary populations also offer the potential for increased yield gains through a combination of natural and artificial selection. Given the widespread adoption of the barley EPs in Iran, our hypothesis was that one of the reasons could be in their agronomic superiority. The study did not fully validate this hypothesis, although it demonstrated the superior stability of the EPs. However, the absence of significant differences for the agronomic traits we measured suggested that superior feed quality, the increased biodiversity, better resilience against unpredictable conditions, better adaptation to low- or no-input conditions and the seed sovereignty could be possible alternative hypotheses explaining the adoption—hypotheses that can be tested in further studies.

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