



Selection of Tunisian Local Pea Populations for the Development of a Composite Variety

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Abstract

This study aims to address the challenges of agricultural sustainability and food security in arid regions by focusing on the genetic improvement of 12 pea (*Pisum sativum* L.) populations (11 local and 1 commercial) cultivated in the southern arid zones of Tunisia. Conducted over four years (2016–2019), this breeding program sought to develop composite varieties with improved yield, quality, and resilience to environmental stresses. Classical breeding methods, augmented by molecular tools (SSR and ISSR markers) and statistical approaches (selection indices), were used to evaluate genetic diversity and identify promising genotypes. A thorough multi-marker analysis, integrating genetic, morphological, and biochemical data, classified the populations into two distinct groups: white-flowered populations (P3, P5, P7), characterized by high yields and protein content, and purple-flowered populations (P2, P6, P8, P9), distinguished by their high seed productivity and rich antioxidant content. These findings highlight the successful integration of traditional breeding techniques and molecular approaches, providing a robust foundation for developing resilient pea varieties tailored to arid environments.

1. INTRODUCTION

Pea (*Pisum sativum* L., $2n = 14$) is a leguminous crop of considerable nutritional and agronomic importance, primarily cultivated in temperate and subtropical regions across the globe (Smýkal et al., 2012; Gudadinni et al., 2017). Despite its relatively limited global cultivation compared to other pulses, pea is valued for its edible tender pods, foliage, and both immature and mature seeds. The species is widely recognized for its rich nutritional profile, containing high levels of proteins (ranging from 23% to 33%), carbohydrates (approximately 42.65%), essential minerals, vitamins, dietary fiber, and diverse antioxidant compounds (Koivunen et al., 2016; Singh et al., 2017). Its high protein content, coupled with a favorable amino acid balance and low levels of trypsin inhibitors, makes pea a valuable food source suitable for individuals across all age groups (Aysh et al., 2013).

Beyond its nutritional benefits, *P. sativum* plays a crucial role in sustainable agriculture. Like other legumes, it contributes to soil fertility through symbiotic atmospheric nitrogen fixation, thereby supporting productivity in low-input and ecologically sustainable farming systems (Ludidi et al., 2007; Tulbek et al., 2017).

In recent years, the global demand for pea has increased due to population growth and shifts in dietary preferences. This has underscored the need to develop high-yielding genotypes adapted to a variety of agro-ecological zones, including the challenging arid environments of southern Tunisia. Breeding efforts in such regions require a comprehensive understanding of the genetic variability available within local and introduced germplasm. Assessment of genetic diversity provides a foundation for effective selection and hybridization strategies aimed at improving yield and stress resilience (Afreen et al., 2017).

Heritability estimates are critical in plant breeding, as they inform the degree to which

desirable traits are transmitted from one generation to the next, thus aiding the identification and selection of elite genotypes (Addissu, 2011). According to Falconer (1981), the genetic basis of trait variability is central to the success of selection programs. In parallel, correlation analysis of agronomic traits, especially those related to yield, provide insight into the extent of trait interdependence and allows for efficient indirect selection (Ahmad et al., 2014). For instance, studies have reported strong genotypic and phenotypic variability in traits such as plant height, number of pods per plant, and seed yield (Kumar et al., 1999; Guleria et al., 2009). Others have emphasized the importance of traits like days to flowering, pod length, number of seeds per pod, and 100-seed weight as key indicators of yield potential (Mital and Verma, 1991; Jaiswal et al., 2015; Tesfaye, 2021).

Despite the importance of pea cultivation in local farming systems, there are no varieties adapted to irrigated cultivation in arid southern Tunisia. Current breeding efforts have largely overlooked the rich genetic resources preserved by local farmers. Therefore, developing a composite variety from locally adapted populations represents a novel and strategic approach that simultaneously promotes genetic conservation and the creation of resilient, high-performing cultivars suited to marginal environments. In this context, the present study aims to evaluate the genetic variability, heritability, and trait associations among 12 pea (*Pisum sativum* L.)

populations cultivated in arid environments of southern Tunisia, using agro-morphological, biochemical, and molecular (SSR and ISSR) data. Our main objective is to identify and select promising genotypes for the development of composite varieties adapted to irrigated arid zones. We hypothesize that (i) significant genetic diversity exists among local populations, (ii) selection indices can effectively improve yield-related traits, and (iii) a multi-marker approach will provide a more robust basis for selection than morphological traits alone.

2. MATERIAL AND METHODS

2.1. Plant material

A breeding program was initiated at the Arid Regions Institute (IRA) to conserve and improve local pea (*Pisum sativum* L.) germplasm collected from various arid regions of Tunisia. The study involved 11 local populations and one commercial variety ('Alexandra') used as a reference (Table 1, Fig. 1). These populations originated from different ecological zones: the "Mareth" region, a coastal oasis under a Saharan Mediterranean climate, benefits from strong maritime influence, creating a distinct "oasis effect" characterized by reduced wind, lower temperatures, and higher humidity compared to the surrounding desert (Sghaier, 1996). The "Ksar Jwamaa" and "Ksar Elhallouf" areas, located in the mountainous region of Béni Khedache at altitudes between 350 and 600 meters, are protected from maritime and desert influences by natural escarpments, resulting in

Table 1. Characteristics of the origin areas of local pea genotypes collected from the arid regions of southern Tunisia.

Code	Population name	Collection site	Province	Latitude (N)	Longitude (E)	Altitude (m)
P1	Alexandra	-----	G.S.N (SEMENCES TUCOD)	---	---	---
P2	P1001	Ksar Jowamaa, Beni khedach, (Continental)	Medenine	33°15' 33°15'	10°11' 10°11'	506 506
P3	P3001	Ksar hallouf, Beni khedach (Continental)	Medenine	33°29'	10°15'	386
P4	P5001			33°29'	10°15'	386
P5	P6001			33°29'	10°15'	386
P6	P6003			33°29'	10°15'	386
P7	P7001	Mareth Oasis (Coastal)	Gabes	33°37'	10°16'	48
P8	P7002			33°37'	10°16'	48
P9	P9001			33°37'	10°16'	48
P10	P9002			33°37'	10°16'	48
P11	P9003			33°37'	10°16'	48
P12	P1002			33°37'	10°16'	48

higher rainfall levels (Boubakri, 1990) (Fig. 2).



Fig. 1. Photo illustrating the seeds of different populations collected from various arid regions.

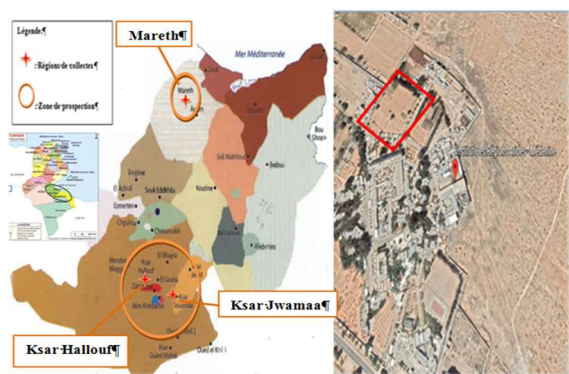


Fig. 2. Map of the seed collection regions for pea populations and satellite image of the experimental plot at the Laboratory of Arid Agriculture and Oasis Crops, Arid Regions Institute of Medenine.

The experimental work was carried out over three growing seasons (2015–2018) at the IRA experimental station in Medenine (33°29'57.80"N, 10°38'32.96"E; 16 m altitude). Twelve genotypes were arranged in a randomized complete block design with three replications, with twelve plants per genotype spaced 40 cm apart within and between rows (Fig. 3). The plants were irrigated twice a week and fertilized with sheep manure incorporated into the soil before sowing, followed by applications of mineral fertilizer (NPK 20-20-20) at seedling emergence, pre-flowering, and pod formation stages.

2.2. Agro morphological Characterization

The agro morphological characteristics of 12 pea populations were assessed using 10 randomly selected plants per population. A total of 48 morphological traits (quantitative, qualitative, and phenological) were evaluated, using the UPOV TG 7/9 descriptor for *Pisum*

sativum L. (UPOV, 1990). The results were previously published by Mohamed et al (2019).

2.2.1. Selection of Elite Lines and Conservation of Diversity

2.2.1.1. Development of Initial Lines

Twelve populations collected from local farmers were characterized and described according to the UPOV pea descriptor (1998). These were considered a base collection of *Pisum sativum* genetic resources from the arid regions of Tunisia, preserved *ex situ* to ensure the long-term conservation of genetic variation for scientific purposes and as a foundation for future selection. Given the study's objectives, a recurrent selection strategy was adopted. In each generation, the best-performing descendants were selected in limited numbers from the evaluated lines. These individuals constituted the genotypes for the following selection cycle. The mixture of top-performing lines from the studied populations served as the nucleus for the development of a composite variety. The goal of this phase was to conduct index-based selection within these twelve populations to obtain genetically homogeneous elite lines that would serve as the progenitors for composite varieties adapted to the arid farming systems of Tunisia.

2.2.1.2. Selection of Elite Lines

The best individuals, identified based on the highest selection index values, were sown in rows and divided into three blocks. Individuals within the same row were compared to one



Fig. 3. Photo of the trial conducted during the 2017–2018 season.

another to assess their level of homogeneity. Selections and eliminations were made based on this homogeneity and the relevance of traits for the selection objectives (e.g., yield, earliness). Once a satisfactory level of uniformity was achieved in the selected lines over successive years, measurements and observations were

recorded on target traits used for calculating the selection index. To choose the best-performing lines that met the breeding objectives, an evaluation table was developed. This table included the following traits: grain yield, number of nodes, stems, and pods, as well as emergence date, flowering date, maturity date, and plant height. The preselected pea populations were evaluated throughout the 2019 season to test their potential for creating composite varieties, which would later be evaluated on-farm. Only those lines meeting the selection criteria were retained using the same evaluation process.

2.2.1.3. Purification Techniques for Selected Elites

The twelve populations were collected based on the preferences and criteria used by local farmers. For each individual plant, a selection index was calculated using the formula: $I = b_1x_1 + b_2x_2 + \dots + b_nx_n$ (Baradat et al., 1994), where b_1 to b_x are the weighting coefficients assigned to traits x_1 to x_n . The weighting coefficients assigned to each trait in the selection index were determined based on expert knowledge of local agronomic conditions, breeders' experience, and previous studies (e.g., Loumerem, 2004; Baradat et al., 1994), with a focus on traits most predictive of yield and stress tolerance under arid environments. The determination of these weights depended both on a reasonably accurate estimation of the genetic value of each trait and on the relative economic importance attributed to each one. In other words, the weighting values were defined arbitrarily but rationally, based on the preferences of farmers and consumers, as well as agricultural and genetic theory and practice (Loumerem, 2004). For calculating the selection indices, target traits were combined with associated predictive traits to improve the accuracy of genetic value estimates (Loumerem, 2004). The primary traits used included grain yield, number of nodes, stems, and pods, as well as emergence, flowering, and maturity dates, and plant height.

The selection index was structured with the following weighting distribution:

-70% of the total weight was assigned to grain yield components (30% seed weight per plant, 20% 100-seed weight, 10% pod weight per plant, 6% number of pods per plant, and 4% number of seeds per pod);

-12% was attributed to phenological stages (4% emergence date, 4% flowering date, and 4% pod maturity date);

-18% was allocated to morphological traits (9% plant height, 4% number of nodes, and 5% number of stems).

Consistency across years was assessed by tracking selection index values over four growing seasons. The observed increase in average index values and reduction in variability (difference between minimum and maximum) were used as indicators of genetic gain and trait stability. This approach aligns with previous methods for evaluating repeatability in multi-year selection trials (Loumerem, 2004).

2.3. Molecular Characterization

The molecular characterization of local pea (*Pisum sativum* L.) accessions was conducted using both SSR and ISSR markers to evaluate their genetic diversity and structure. The selected markers were chosen based on their documented polymorphism and reproducibility in previous studies on *Pisum sativum* (e.g., Mohamed et al., 2019; Mohamed et al., 2022). Priority was given to markers that produced clear, scorable bands and provided broad genome coverage to maximize discrimination among populations. A total of eight SSR markers and fifteen ISSR markers were applied to 12 local pea accessions collected from the arid regions of southern Tunisia. The combined use of SSR and ISSR markers proved highly effective in revealing the genetic variability among the accessions and provided valuable insights for future breeding programs focused on enhancing pea adaptation to arid environments. These results were previously published in an earlier article (Mohamed et al., 2019; Mohamed et al., 2022).

2.4. Biochemical characterization

In this study, twelve pea (*Pisum sativum* L.) populations collected from arid regions of southern Tunisia, differing in flower and seed coat color, were analyzed to assess their protein content, phenolic composition, and antioxidant activity. Biochemical analyses were carried out using three replicates per population. The selected traits were chosen based on their nutritional importance and their potential role as indicators of physiological performance and environmental adaptation in arid conditions, in line with previous studies (Mohamed et al., 2022). The results of this investigation were

previously published in an earlier article (Mohamed et al., 2022).

2.5. Statistical analysis

Following the selection of the most promising populations, this choice was validated through a multimarker analysis that integrated genetic, morpho-agronomic, and chemical data from the local pea populations. A comprehensive dendrogram was generated using the R statistical software to visualize the clustering and relationships among populations.

3. RESULTS

3.1. Interpretation of Selection Results

The selection index values (minimum, maximum, and average) for each population over the three years of selection are presented in Table 2. In 2016, plant lines that showed greater uniformity in the evaluated traits were preferred, and a higher number of individuals were selected from those lines. However, in 2017 and 2018, a much stricter selection was applied, with entire rows being eliminated if they contained individuals showing heterogeneity in the selected traits. The average selection index values increased year by year, clearly reflecting the improvement achieved in the traits contributing to the indices. Additionally, the difference between maximum and minimum values progressively decreased, indicating an increase in the homogeneity of individuals selected to form the populations. These results demonstrate that index-based selection is effective in improving local pea germplasm. The outcomes obtained at the IRA experimental station in Medenine are promising. Overall progress was observed across the three

years of selection for all target traits. However, it was deemed useful to complement these results with early testing. Indeed, the ultimate goal of this breeding work is to develop high-performing material that is both highly productive and tolerant to the environmental stresses of arid zones. The methods used to achieve this are often time-consuming, so early tests are being explored to accelerate the improvement process and shorten the breeding cycle. Early testing, using target traits as predictive parameters, could serve as an effective tool for evaluating the progress made. Populations that were excluded from the 2019 evaluation (e.g., P1, P4, P10, P11, and P12) had shown lower selection index values and greater intra-population variability during earlier selection cycles. As a result, only populations meeting the selection criteria for uniformity and agronomic potential were advanced for further testing in 2019. Based on our results, two composite varieties can be established from the populations with the highest index values: one white-seeded (comprising populations P3, P5, and P7), and one purple-seeded (comprising populations P2, P6, P8, and P9). These populations are characterized by early flowering and maturity, as well as high yield potential. In 2019, a test was conducted on these most promising lines to assess their stability in terms of morphological parameters and nutritional qualities. The 2019 selection index results (Table 2, Fig. 4) were consistent with previous findings. The test showed an increase in index values, confirming the growing uniformity within these populations and validating our selection approach.

Table 2. Selection index of plants retained during the years 2016, 2017, 2018, and 2019.

Populations	Selection index of selected plants											
	Year 2016			Year 2017			Year 2018			Year 2019		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
P1	3,02	5,60	3,55	2,95	4,38	3,69	2,85	4,36	4,33	---	---	---
P2	4,14	8,25	4,19	3,69	5,44	4,69	4,18	5,97	5,93	5,82	6,32	6,21
P3	3,08	5,08	4,13	3,65	5,67	4,13	3,09	5,24	4,58	4,54	4,98	4,71
P4	3,26	4,32	3,35	2,53	4,26	3,42	2,64	4,13	3,58	---	---	---
P5	3,51	5,92	3,54	3,02	5,26	4,12	3,66	4,65	4,46	4,21	4,78	4,68
P6	2,71	5,56	3,97	3,44	5,24	4,36	3,24	4,64	4,40	4,32	4,62	4,51
P7	4,11	7,05	4,16	3,72	5,89	4,67	4,15	5,85	5,60	5,72	6,11	5,87
P8	2,92	6,05	4,13	3,55	5,08	4,36	3,43	4,95	4,72	4,79	5,12	4,91
P9	3,60	5,83	3,96	3,50	5,20	4,31	3,09	4,97	4,76	4,81	5,23	5,09
P10	2,99	5,57	3,08	2,25	3,94	3,15	2,42	4,44	4,36	---	---	---
P11	2,36	6,07	2,74	1,93	3,89	2,78	3,17	4,41	4,26	---	---	---
P12	2,22	5,27	2,76	2,61	4,37	3,43	3,08	4,39	4,21	---	---	---

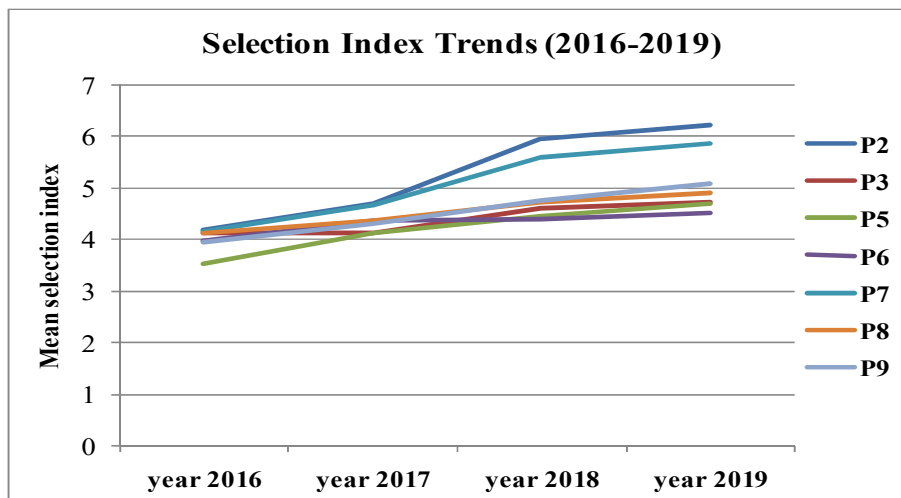


Fig. 4. Trends in mean selection index values for elite populations across four selection cycles (2016–2019).

3.2. Selection Based on Multi-Marker Analysis

To classify the local pea populations based on the full set of available genetic (SSR and ISSR), morpho-agronomic, and biochemical data, we calculated the Gower distance, which is a dissimilarity index suitable for combining both quantitative and qualitative variables. This analysis was performed using the R software. Based on the generated matrix, a comprehensive dendrogram was constructed (Fig. 5). This analysis was conducted to validate our index-based selection strategy. According to the dendrogram, two main groups of populations can be distinguished: Group 1 includes populations P1, P3, P5, P7, and P12, while Group 2 comprises populations P2, P4, P6, P8, P9, P10, and P11.

Notably, these Tunisian local populations do not cluster according to their geographical origin. Instead, they are grouped based on morphological traits: all populations in Group 1 are characterized by white flowers, whereas those in Group 2 have purple flowers. This finding highlights the limitation of relying solely on morphological traits to assess the genetic variability of Tunisian pea populations. Therefore, we incorporated biochemical and molecular characterizations. The multi-marker analysis of genetic diversity in these local populations aligns with the outcomes of our index-based selection. This clustering pattern reflects a high degree of consistency with the selection index-based classification, confirming the robustness of our multi-marker approach. The dendrogram supports our choice of

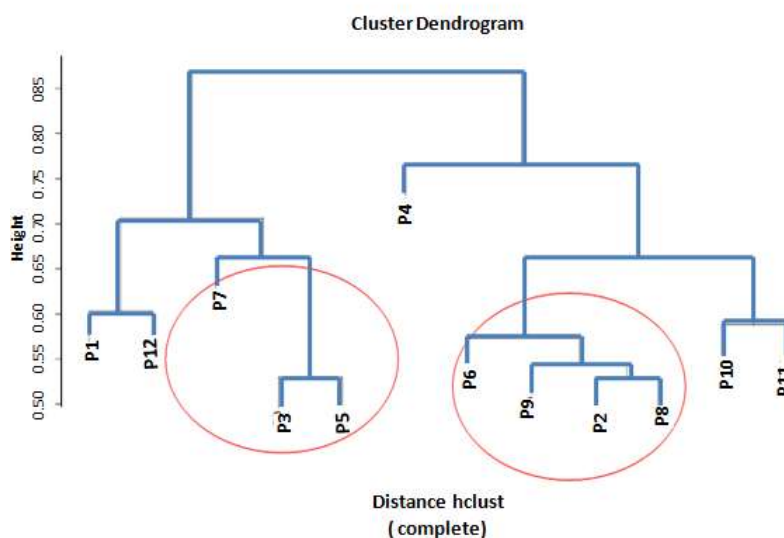


Fig. 5. Global dendrogram generated using the R software based on all genetic, morpho-agronomic, and biochemical data of local Tunisian pea populations.

populations for the development of two composite varieties. Indeed, it appears that these populations share more biochemical and genetic features than morphological ones. Each marker system captures different aspects of genetic variability, which may explain inconsistencies often observed in genetic diversity studies. Nevertheless, despite the strong capacity of molecular markers to detect variability and genetic relationships, they should not be regarded as substitutes for traditional agro morphological descriptors. All marker systems should be seen as complementary tools that, together, enhance our understanding of diversity in local pea populations, thus supporting their use in breeding programs for arid regions.

4. DISCUSSION

To ensure agricultural sustainability and food security, one of the main responsibilities of plant breeders is to develop elite varieties with high productivity and strong tolerance to both biotic and abiotic stresses. This task depends on the rich genetic diversity found in germplasm conserved in gene banks (Johal et al., 2008; McCouch et al., 2013; Byrne et al., 2018). These conserved resources serve as carriers of genetic and biological information with real or potential utility value and are considered the foundation for crop genetic improvement (Li et al., 2015; Liu et al., 2018).

Currently, no pea varieties specifically selected for irrigated areas in arid regions are available in the local seed market. The present work is part of a breeding program aiming to develop new composite varieties of peas that are well adapted to the soil and climatic conditions of arid zones. Various constraints may affect the selection and production of peas in these regions. Therefore, several factors must be considered, including varietal performance, yield, quality, resistance to pests and diseases, and tolerance to biotic and abiotic stresses (Ali et al., 2007; Sardana et al., 2007; Smýkal et al., 2008a, b; Sarikamis et al., 2010; Handerson et al., 2014).

Today, selection criteria have significantly evolved. With the use of molecular markers, new perspectives have emerged for breeders. Using classical breeding methods, pea breeders worldwide have achieved significant progress in improving yield, quality, and stress resistance. These traditional approaches are primarily used to develop new genetic varieties through hybridization between contrasting parental lines. However, conventional breeding methods

have certain limitations, such as long breeding cycles and low efficiency. Moreover, it is challenging to combine multiple useful genes into a single elite genotype and effectively track them in subsequent breeding programs (Kenei et al., 2005; He & Zong, 2011). Thus, the use of index selection in our study demonstrates that this method is effective in improving yield-related traits in peas cultivated in arid regions. Our results are consistent with findings from other legume improvement programs in arid zones. For instance, Loumerem (2004) demonstrated the effectiveness of index selection in enhancing grain yield and earliness in pearl millet under Tunisian dryland conditions. Similarly, Sallam and Moursi (2015) reported substantial progress in faba bean breeding through multi-trait selection under abiotic stress. In chickpea, Kumar et al. (2011) and Jukanti et al. (2012) highlighted the importance of integrating biochemical and molecular markers to improve drought tolerance and nutritional quality. These parallels support the robustness of our multi-marker selection strategy for pea and validate its applicability in breeding programs targeting resilience and performance in arid agroecosystems.

The role of biotechnology in pea breeding can also be applied through molecular selection methods, including genome-assisted selection, marker-assisted selection, and genetic engineering. These techniques have seen significant innovations and advancements over the past three decades (Xu et al., 2012; Poland, 2018). Genome selection has garnered considerable attention, as designing desirable plants based on markers and associated genetic information is increasingly feasible (Chen et al., 2018; Scheben & Edwards, 2018; Sharma et al., 2020, Azam et al., 2024). This approach relies on the development of high-density genetic linkage maps based on SSR, SNP markers and important agronomic traits. For example, in 2008, 25 pea varieties were included in the list of recommended cultivars for farming in the Czech Republic, having been selected through combined morphological, biochemical, and molecular evaluations (Smýkal et al., 2008). Furthermore, additional association mapping work was conducted on 285 USDA core pea collections using 137 DNA markers, including SSR, RAPD, and SCAR. Kwon et al. (2012) identified three subgroups within this collection and discovered 10 markers associated with seed micronutrients, 11 with disease/pest resistance, 42 with morphological/agronomic traits, 4 with

phenology, and 10 with yield. Other studies have combined morphological and molecular characterizations to identify markers linked to important agronomic traits or abiotic stress resistance. These studies have laid the groundwork for marker-assisted selection in pea breeding (Smýkal & Konečná, 2014; Cheng et al., 2015; Diapari et al., 2015; Liu et al., 2017).

The development of composite varieties from genetically diverse, locally adapted pea populations offers a strategic advantage for integration into decentralized seed systems. Such varieties, due to their broad genetic base, are more resilient to environmental variability and can be readily multiplied and selected by farmers. This approach also aligns with participatory breeding models, where farmers are actively involved in the selection and maintenance of varieties, ensuring better adoption, adaptability, and sustainability under real on-farm conditions. As a result, these composite varieties have strong potential to strengthen local seed sovereignty and enhance food security in arid regions.

5. Conclusions

It can be concluded that it is essential to characterize genetic material in a comprehensive and accurate manner for any crop. The results of the multi-marker analysis conducted using all available genetic (SSR and ISSR), morpho-agronomic, and biochemical data for pea allowed us to identify two distinct population groups. The first group includes populations with white flowers (P1, P3, P5, P7, and P12), while the second consists of populations with purple flowers (P2, P4, P6, P8, P9, P10, and P11). This analysis is consistent with the results obtained through index selection, as it supports the selection of populations for the development of two composite varieties. The first composite variety, with white flowers, is composed of populations P3, P5, and P7, which are characterized by high yields and high protein content. The second composite variety, with purple flowers, consists of populations P2, P6, P8, and P9, characterized by high seed production and rich in antioxidants and phenolic compounds. Therefore, the genetic characterization of these populations provides an important theoretical foundation, which can lead to a better understanding and utilization of these resources. Future work will focus on evaluating the selected composite populations under real farming conditions through on-farm trials in collaboration with local producers. This

participatory approach will help assess adaptability, farmer preference, and seed system integration potential. In parallel, efforts will be made to initiate the registration process for the most stable and high-performing composite lines to ensure their formal release and wide dissemination. By harnessing local genetic resources and applying multi-dimensional selection strategies, this study contributes to the development of resilient pea varieties that support food security and climate adaptation in water-limited environments, offering a scalable model for sustainable agriculture in arid regions

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