American Journal of Agriculture and Forestry
2026, Vol. 14, No. 2, pp. 120–132
https://doi.org/10.11648/j.ajaf.20261402.15

Research Article

Assessment of Heterosis and Inbreeding Depression on Agronomic Traits in TPS-derived Potato Hybrids Under Northern Pakistan Climatic Conditions

Bilal Ahmed Khan[1, *], Naushad Ali[1], Sardar Ali[1], Mehboob Ahmed[2], Muhammad Tehseen Zaheer Tanoli[2], Maria Saleem[2], Hassan Tariq[3], Muhammad Kifayatullah[3]

[1]Department of Plant Breeding and Genetics, University of Haripur, Haripur, Pakistan
[2]Hazara Agriculture Research Station, Abbottabad, Pakistan
[3]Seed Potato Research and Multiplication Farm, Battakundi, Pakistan

Abstract

True Potato Seed (TPS) breeding offers a promising pathway to develop high-yielding, disease-resistant, and climate-resilient potato hybrids, contributing to food security and sustainable agriculture in regions such as Pakistan. This study evaluated heterosis and inbreeding depression in eight potato hybrids derived from true potato seed under climatic conditions of Northern Pakistan. A randomized complete block design was employed with three replications at Battakundi and Abbottabad during 2022–2024. Analysis of variance revealed that all the genotypes had showed highly significant differences ($P < 0.01, 0.05$), reflecting substantial genetic variability. High estimates of relative heterosis (64.10%), heterobeltiosis (54.58%) for the number of marketable tubers per plot, and standard heterosis (48.64%) for their weight revealed strong hybrid vigor. Negative inbreeding depression ranging from –0.81% to –349.7% suggested superior hybrid performance over parental cultivars. Positive correlations among yield and yield attributed traits provided greater opportunities for selection in potato breeding. Results concluded that among the tested combinations, Sarpomira × Roko and Kuroda × Burna demonstrated significant positive heterotic effects for yield traits and desirable negative heterosis for late blight incidence, identifying them as promising candidates for future hybrid release. Overall, the findings highlight TPS breeding as a viable approach for enhancing productivity and resilience of potato crops in high-altitude and similar agro-ecological regions.

Keywords

True Potato Seed, Heterosis, Inbreeding Depression, Sarpomira × Roko, Kuroda × Burna

1. Introduction

The potato (*Solanum tuberosum* L.) an annual herbaceous crop, contributes significantly to food security because of its high nutritional value, adaptability to various environments, and wide range of uses [1]. They are recognized as a valuable source of essential nutrients, mainly vitamin C, antioxidants, dietary fiber, and several important micronutrients like iron,

---

[*]Correspondence: Bilal Ahmed Khan (bilalahmed_pbg@yahoo.com)

Received: 2 November 2025; **Accepted:** 18 November 2025; **Published:** 28 April 2026

potassium, vitamin B6, and folate, all of which contribute significantly to human nutrition and health [2]. According to the most recent statistics, global potato production reached approximately 383 million metric tons in 2023, cultivated over an estimated 18 million hectares, reflecting the crop's continued significance in global food systems [3]. In Pakistan, potatoes were cultivated on about 0.341 million hectares during the 2023–24 growing season, yielding approximately 8.434 million tons, with Punjab province contributing the majority of national output [4]. These figures underscore the importance of potato cultivation in ensuring both global and national food security.

Heterosis, or hybrid vigor, describes the phenomenon in which first-generation (F_1) hybrids derived from the crossing of genetically diverse parental lines exhibit superior performance as compared to parental cultivars for major agronomic characters such as yield, growth rate, and reproductive efficiency [5, 6]. Recent studies have reaffirmed the pivotal role of heterosis in potato breeding, recognizing it as a fundamental mechanism for developing high-yielding, disease-resistant, and economically viable hybrids [7, 8]. Heterosis can be either relative heterosis, heterobeltiosis, and standard heterosis, reflecting different genetic means, like over-dominance, partial to complete dominance, epistasis, or their joint interactions [1, 9]. Inbreeding depression refers to the reduction in vigor, yield, fertility, and overall performance resulting from mating among genetically related individuals, which leads to decreased heterozygosity and the expression of deleterious recessive alleles [10, 11]. This phenomenon is particularly critical in clonally propagated crops such as potato, where continuous vegetative propagation exacerbates the accumulation of harmful mutations [12]. In auto-tetraploid potatoes ($4x = 48$), inbreeding depression is largely attributed to the masking and subsequent expression of deleterious alleles within the complex polyploid genome [13]. To mitigate these challenges, the True Potato Seed (TPS) breeding approach aims to transition potato improvement from the auto-tetraploid to the diploid ($2x = 24$) level, where genetic architecture is simplified, and deleterious alleles can be more effectively purged through controlled inbreeding [14, 15].

In Pakistan, potato crop is widely grown under diverse environmental conditions, from the irrigated plains of Punjab to the cooler highlands of Khyber Pakhtunkhwa and Gilgit-Baltistan, demonstrating broad adaptability and regional versatility [16]. Despite the favorable agro-ecological potential for large-scale production, Pakistan continues to face significant challenges in fulfilling its domestic seed potato demand, relying heavily on expensive annual imports. These constraints stem from the limited availability of true to type, disease-free, and approved seed, slow release of superior cultivars, and suboptimal crop management and storage practices [17, 18]. To overcome these barriers, the True Potato Seed (TPS) approach has gained growing attention as a cost-effective and sustainable breeding strategy, particularly in northern Pakistan climatic conditions. TPS technology provides several advantages, including enhanced disease resistance, greater genetic diversity, and improved yield potential, thereby offering a viable alternative to conventional clonal propagation and reducing dependence on imported seed potatoes [19, 20]. Adoption of TPS-based breeding and localized seed production systems could substantially strengthen the resilience and self-sufficiency of Pakistan's potato industry.

Therefore, the present study was undertaken to evaluate and select promising cross combinations of potato hybrid in at earlier stages with a primary focus on assessing heterosis and inbreeding depression. The research specifically aims to identify and select potato hybrids exhibiting superior performance for key agronomic traits, including tuber yield, earliness and disease resistance especially for Phytophthora infestans, and yield-attributing characteristics for the high-altitude conditions of Northern Pakistan. Furthermore, a correlation was studied in order to examine the interaction among tuber yield and its contributing characters, aiming to identify key yield determinants that can serve as reliable selection criteria in future breeding programs. The results from this study can lead towards the betterment of potato breeding strategies, improving productivity, adaptability, and food security in challenging production environments.

2. Materials and Methods

2.1. Study Site Overview

The research was carried out at two distinct locations: the Seed Potato Research and Multiplication Farm, Battakundi (34.9316° N, 73.7743° E), and the Hazara Agricultural Research Station, Abbottabad (34.1688° N, 73.2215° E), during 2022–2024 cropping seasons. The experimental sites are situated at elevations of 1,256 m and 2,812 m, respectively. These two regions obtain considerable annual precipitation, averaging 1,500 mm in Battakundi and 1,532 mm in Abbottabad. Average annual temperatures are approximately 3.9°C in Battakundi and 15.9°C in Abbottabad. Soil analyses revealed somewhat acidic conditions, with pH values of 6.3 and 5.5 and at the respective sites. Battakundi, located in the upper Kaghan Valley of Khyber Pakhtunkhwa, represents one of Pakistan's major off-season potato-producing zones. Owing to its cool summer climate, the region offers highly favorable conditions for potato cultivation at a time when the lowland plains of the country experience temperatures unsuitable for the crop.

2.2. Description of Experimental Material

The parental cultivars utilized in this study for crossing are Roko, Sarpomira, Kuroda, Cardinal, and Burna. These genotypes were strategically chosen for their quantitative and qualitative attributes that are vital for commercial potato improvement. Selection was primarily based on characteristics such as high tuber yield, resistance to disease (late blight), early maturity, a greater proportion of marketable tubers (>55 mm), and attractive red skin color. To produce genetically diverse hybrid combinations, crosses were carried out among these distinct parental lines at the Battakundi, in 2022, aiming to create a broad spectrum of heritable variability.

Following successful pollination, potato berries (F_1 fruits) containing True Potato Seed (TPS) were collected. The extracted true seeds were sown in controlled nursery conditions to raise the F_1 generation, resulting in genetically diverse hybrid plants. Among these F_1 progenies, superior individuals were identified and selected based on key evaluation parameters such as seedling vigor, viability, tuber yield potential, and favorable agronomic traits, including earliness, disease resistance, and market-preferred tuber characteristics (size >55 mm, uniform shape, and skin color). Tubers harvested from the selected F_1 plants served as the planting material for establishing the F_2 generation, which was propagated vegetatively for further performance evaluation under varying environmental conditions.

The experimental material used in this investigation comprised seven different cross combinations, five parental cultivars, and one check hybrid. The hybrid combinations included: Kuroda × Burna, Cardinal × Burna, Kuroda × Roko, Sarpomira × Roko, Cardinal × Roko, Roko × Sarpomira, and Kuroda × Sarpomira. The hybrid Sarpomira × Kuroda (S × K) was used as a check due to its high yield potential, tolerance to diseases, and early maturity, making it a standard for comparative analysis. The pedigree details of the parental lines, including their genetic origin, breeding background, and distinct traits, are given in Table 1, providing insights into the genetic miscellany and potential performance of the derived hybrids.

2.3. Experimental Design and Layout

A randomized complete block (RCB) design with three replications was used to ensure statistical precision and to minimize the influence of environmental variability among treatments. This experimental design was chosen to enhance the reliability, reproducibility, and accuracy of the obtained results by effectively accounting for field heterogeneity. The planting arrangement was standardized with a row spacing of 0.75 m and an intra-row spacing of 0.25 m to achieve optimal plant density. Each replicated plot consisted of two rows containing a total of 24 plants, occupying a plot area of 4.5 m². The tubers used for planting were derived from True Potato Seed (TPS) seedlings raised in a nursery during the early growth phase. The incorporation of replication and randomization was essential for minimizing experimental bias, controlling unforeseen environmental effects, and improving the precision and validity of the experimental findings.

2.4. Management of Experimental Field

The experimental fields were prepared following standard plowing procedures and aligned according to the pre-defined layout. Healthy, uniformly sprouted seed tubers were planted manually to ensure uniform crop establishment. Fertilizer inputs consisted of Di-ammonium phosphate (DAP), sulfate of potash (SOP), and urea, applied at rates of 220, 260, and 180 kg ha^{-1}, respectively. Throughout the cropping period, recommended agronomic practices including regular irrigation, weed control, and other management operations were carried out systematically. Manual harvesting was employed to ensure precise and consistent data recording. Additional cultural practices such as hoeing, spraying, and weeding were performed uniformly and on schedule to promote optimal plant growth and vigor. To facilitate an accurate evaluation of natural disease incidence, particularly for late blight and other prevalent potato diseases, no fungicides or insecticides were used during the experiment. This strategy enabled an unbiased assessment of genotypic resistance or tolerance under natural field conditions and ensured the authenticity of disease response observations.

2.5. Data Collection

2.5.1. Phenological Parameters

The number of days from planting until 50% of the plants emerged was recorded as days to 50% emergence [21]. The progression of disease symptoms was observed and documented weekly [22]. Typical late blight symptoms were identified as light to dark brown lesions on the upper leaf surface, accompanied by a downy white to grayish fungal growth (3–5 mm wide) on the underside of the leaves. The disease incidence (%) was computed using the following formula [22].

$$\text{Disease Incidence (\%)} = \text{Number of Infected}\,\frac{\text{Plants}}{\text{Number}}\,\text{of Total Plants} \times 100 \tag{1}$$

The disease severity and resistance level were evaluated using the 1–9 rating disease scale (Table 2), as recommended by [22]. The mean values obtained from these assessments were used for subsequent statistical analysis.

2.5.2. Growth Parameters

Data on growth attributes including plant height (cm), leaf area per plant (cm²), and number of stems per plant, were recorded during the experimental period. The height of seven randomly selected plants from each plot was calculated with the help of measuring rod, taken from the soil crust to the tip of the main stem at the stage of 50% flowering. Likewise, the number of stems and leaf area per plant were determined from the same set of randomly selected plants at the same growth stage [21]. The average values of these observations were then computed and used for subsequent statistical analysis.

2.5.3. Tuber Yield and Yield Related Parameters

The harvested potato tubers were classified into four size categories: large (>75 g), medium (39–75 g), small (25–38 g), and very small (<25 g), following the classification method [21]. Tubers from defined types were counted and separately weighed to ensure precise data recording. Particular emphasis was placed on the number and weight of marketable tubers per plot (>25 g). Tubers that were healthy, disease-free, and weighed 25 g or more were categorized as marketable, whereas those that were damaged, diseased, pest-infested, or weighed less than 25 g were classified as unmarketable, as outlined by [21]. The weight variation among seed-sized tubers within each category was maintained within ±2.5 g to ensure uniformity. Additionally, the total number and total weight of tubers per plot were noted, and tuber yield was changed to t ha^{-1}, and used the given formula to convert yield in to t ha^{-1} [23].

$$\text{Tuber Yield}\left(\frac{\text{Tons}}{\text{ha}}\right) = \text{Plot}\frac{\text{Yield}}{\text{Plot}}\text{Area} \times 100 \tag{2}$$

Environmental parameters such as temperature (°C) and relative humidity (%) were also monitored throughout the experiment (Figure 1). Tracking these environmental variables allowed for the assessment of their influence on plant growth, tuber development, and overall yield performance. Such information provides valuable insights for improving management practices and breeding strategies, particularly in optimizing yield potential under varying environmental conditions.

(i) Heterosis

The superior performance of F_1 hybrids compared to their parental averages or individual parent performance is termed heterosis. Specifically, when F_1 progeny outperform the mid-parental mean, it is known as mid-parent or relative heterosis; when they exceed the better-performing parent, it is referred to as better-parent heterosis (heterobeltiosis); and when they surpass a standard or commercial cultivar, it is identified as standard heterosis. The extent of heterotic expression for each trait was also calculated [11].

$$\text{Relative heterosis (\%)} = \text{F1} - \frac{\text{MP}}{\text{MP}} \times 100 \tag{3}$$

$$\text{Heterobeltiosis (\%)} = \text{F1} - \frac{\text{BP}}{\text{BP}} \times 100 \tag{4}$$

$$\text{Standared heterosis (\%)} = \text{F1} - \frac{\text{SP}}{\text{SP}} \times 100 \tag{5}$$

Where,

$$\text{MP} = \frac{\text{P1} + \text{P2}}{2}$$

The magnitude of heterosis (%) was classified into three categories: high (>20%), moderate (10–20%), and low (0–10%), as proposed by [24].

(ii) Inbreeding depression

The decline in vigor, fertility, yield, and on the whole performance that occurs as a consequence of inbreeding within a population. In the present study, the percentage reduction in F_2 performance relative to the average performance of the corresponding F_1 hybrids is considered as inbreeding depression using the formula given below [11].

$$\text{Inbreeding Depression (\%)} = \text{F1} - \frac{\text{F2}}{\text{F1}} \times 100 \tag{6}$$

(iii) t-test

A t-test was conducted to evaluate whether the observed relative heterosis, heterobeltiosis, and commercial heterosis were statistically significant or not. This test was applied to determine if the performance differences between the F_1 hybrids and their respective parental means were statistically meaningful, thereby providing a quantitative measure of the effectiveness and reliability of the hybridization process. The significance of heterotic effects was assessed using the t-test formula as described by [11].

$$\text{t} = \text{F1} - \frac{\text{MP}}{\text{SE}}(\text{F1} - \text{MP})(\text{Relative heterosis}) \tag{7}$$

$$\text{t} = \text{F1} - \frac{\text{BP}}{\text{SE}}(\text{F1} - \text{BP})(\text{Heterobeltiosis}) \tag{8}$$

$$\text{t} = \text{F1} - \frac{\text{SP}}{\text{SE}}(\text{F1} - \text{SP})(\text{Standard heterosis}) \tag{9}$$

Where,

MP is the mid parent values, BP is the better parent and SP is the standard parent.

F_1 is the performance of the hybrid generation.

SE is the standard error.

2.6. Correlation

To assess the interrelationships among key agronomic and yield-contributing traits, a Pearson's correlation analysis was performed. This statistical approach determines both the strength and direction of linear associations between different characters. The Pearson correlation coefficient (r) varies from –1 to +1, where a positive value ($r > 0$) signifies a direct relationship indicating that both variables increase or decrease simultaneously, whereas a negative value ($r < 0$) reflects an inverse relationship, meaning that an increase in one variable corresponds to a decrease in the other. An value close to zero ($r = 0$) indicates little to no linear association between the studied traits [25]. This analysis provides valuable insights into how characteristics such as tuber yield, plant height, number of marketable tubers, disease incidence, and days to maturity interact, thereby guiding selection strategies and breeding decisions aimed at yield improvement and stress tolerance in potato crops.

2.7. Statistical Analysis

The analysis of variance (ANOVA) were used to determine the statistical significance of differences among treatment means within each environment. Statistix 8.1 software was used during the study for different analysis. On the basis of mean performance, and heterosis estimates, genetic variability parameters were computed for different evaluated traits. The Least Significant Difference (LSD) test was also used at the 5% probability level ($P < 0.05$) to compare treatment means and identify statistically significant differences among genotypes. The LSD test, as a post-hoc comparison method, facilitates pair-wise evaluation of treatment means to detect meaningful differences across experimental treatments. Applying these statistical approaches ensures a robust assessment of genotypic performance, trait variability, and trait interactions under different environments, providing reliable insights to chose and develop advanced potato hybrids in breeding programs.

3. Results

3.1. Analysis of Variance

The analysis of variance demonstrated highly significant ($P < 0.01$) differences in F_2 populations for all the evaluated traits at Battakundi, indicating substantial genetic variability within the studied material. Similarly, in the F_1 generation evaluated at Abbottabad, highly significant ($P < 0.01$) differences were also noted among potato hybrids for most traits. However, number of stems per plant, weight of marketable tubers per plot, total tuber weight per plot, and tuber yield per plot exhibited significant ($P < 0.05$) rather than highly significant variation. These results highlight the existence of considerable phenotypic and genotypic diversity among the evaluated hybrids, providing a strong foundation for effective selection and future breeding advancements (Table 3). The potato hybrids derived from True Potato Seed (TPS) exhibited superior mean performance, reflecting substantial variability across yield-related and agronomic traits under different environmental conditions.

3.2. Heterosis (%)

In potato breeding, the expression and popularity of heterosis depend largely on the particular yield-contributing traits under improvement. The results revealed considerable variability in the magnitude of heterosis among different hybrid combinations, reflecting the diverse genetic backgrounds of the parental lines. The observed range of positive relative heterosis (0.91%–64.10%), heterobeltiosis (0.86%–54.58%), and standard heterosis (0.13%–48.64%) demonstrates substantial hybrid vigor across key agronomic traits, particularly those related to tuber yield and plant performance (Table 4).

3.2.1. Heterosis for Phenological Parameters

In potato breeding, negative heterosis is considered advantageous for specific traits such as days to emergence and late blight incidence, as earlier plant emergence and reduced disease infection contribute to early maturity, escape from late-season stresses, and improved crop health and yield stability. Among the tested hybrids, the cross combination Sarpomira × Roko exhibited notable negative heterotic effects for both days to emergence and late blight disease incidence per plot, recording values of –1.58% and –64.7% for relative heterosis, and –8.82% and –75.0% for standard heterosis, respectively. These results suggest that this hybrid possesses rapid early growth and strong disease tolerance, making it a promising candidate for breeding programs focused on early maturity and disease-resistant cultivars (Tables 5 and 6). Interestingly, all other hybrid combinations showed positive relative heterosis for days to emergence, indicating slower emergence compared with mid-parent performance, except for Sarpomira × Roko and the check hybrid (Sarpomira × Kuroda), which both revealed slightly negative relative heterosis (–0.51% and –1.58%, respectively). For late blight incidence, 87.5%, 50%, and 100% of the hybrids exhibited negative relative heterosis, heterobeltiosis, and standard heterosis, respectively. This pattern highlights a general improvement in resistance levels among the hybrid combinations, with several crosses outperforming both parents in disease tolerance. The combinations Roko × Sarpomira, Kuroda × Burna, and Kuroda × Sarpomira displayed the lowest negative heterosis, with relative heterosis, heterobeltiosis, and standard heterosis values of –44.44%, –48.71%, and –16.66%, respectively (Tables 5 and 6).

3.2.2. Heterosis for Growth Parameters

In potato breeding, positive heterotic effects for vegetative growth traits such as plant height, number of stems per plant, and leaf area per plant are considered advantageous, as they enhance canopy development, photosynthetic capacity, and nutrient utilization efficiency, key determinants of biomass accumulation and yield potential**.** The magnitude of relative heterosis (RH), heterobeltiosis (HB), and standard heterosis (SH) varied notably among the hybrid combinations evaluated in this study. The proportion of crosses exhibiting positive heterosis was 100%, 87.5%, and 62.5% for RH; 87.5%, 87.5%, and 25.0% for HB; and 25.0%, 87.5%, and 75.0% for SH for plant height, number of stems, and leaf area per plant, respectively (Tables 5 and 6). The hybrid Kuroda × Sarpomira displayed the highest positive relative heterosis (35.94%) and heterobeltiosis (30.98%), while Kuroda × Burna expressed the maximum standard heterosis (20.04%) for plant height, suggesting its superior vigor and adaptability. For the number of stems per plant, the cross Roko × Sarpomira recorded the highest heterotic response with RH, HB, and SH values of 52.38%, 44.14%, and 44.14%, respectively, indicating its strong vegetative growth and potential for higher tuber initiation sites. Similarly, Sarpomira × Roko showed the most favorable heterotic expression for leaf area per plant, with RH, HB, and SH values of 13.88%, 4.09%, and 22.05%, respectively, highlighting its efficient canopy expansion and enhanced photosynthetic potential.

3.2.3. Heterosis for Tuber Yield (Tons ha^{-1}) and Yield Attributing Parameters

Positive heterotic effects for number and weight of marketable tubers per plot are among the most desirable attributes in potato improvement programs, as they directly influence total yield potential, seed tuber quality, and commercial market value. In the present study, positive estimates of relative heterosis (RH), heterobeltiosis (HB), and standard heterosis (SH) were recorded for the majority of hybrid combinations, indicating enhanced genetic vigor and productivity. Specifically, positive heterosis was observed in 100%, 62.5%, and 75% of the crosses for number of marketable tubers per plot, and in 100%, 50%, and 50% of the crosses for weight of marketable tubers per plot, for RH, HB, and SH, respectively. Among these, the hybrid Sarpomira × Roko exhibited the highest heterotic response for number of marketable tubers per plot with RH (64.10%), HB (54.58%), and SH (40.96%), while Kuroda × Burna showed superior performance for weight of marketable tubers per plot, recording RH (63.76%), HB (44.87%), and SH (48.64%), respectively (Tables 5, 6). The cross combination Sarpomira × Roko displayed the highest positive heterotic effects across all yield-contributing traits, with RH (15.68% and 19.66%), HB (13.87% and 14.25%), and SH (23.41% and 34.75%) for total number and total weight of tubers per plot, respectively. Furthermore, this cross also demonstrated superior heterotic performance for tuber yield per plot, registering RH (20.96%), HB (14.12%), and SH (35.27%). Conversely, the check hybrid Sarpomira × Kuroda showed negative heterotic values for tuber yield per plot (RH = –10.94%, HB = –22.54%, SH = –8.19%), suggesting limited hybrid vigor for this trait under the studied conditions (Tables 5, 6).

3.3. Inbreeding Depression

The reduction in yield, fertility, and productivity in segregating generations due to increased homozygousity and the expression of deleterious recessive alleles. In the present study, all crosses exhibited negative inbreeding depression for most of the measured characters, like days to emergence (–7.19% to –32.95%), number of total tubers per plot (–10.75% to –77.23%), total tuber weight per plot (–19.66% to –63.74%), tuber yield per plot (–19.66% to –63.75%), leaf area per plant (–0.81% to –30.80%), number of marketable tubers per plot (–14.06% to –223.6%), and weight of marketable tubers per plot (–4.23% to –86.66%), except for the hybrids Sarpomira × Roko and Roko × Sarpomira, which displayed marginally positive ID for a few parameters (Table 6). Interestingly, the cross Sarpomira × Kuroda, a check cross produced positive ID values for plant height (1.38%) and number of stems per plant (7.69%), suggesting some degree of heterozygosity retention or favorable gene recombination for these traits. Moreover, 37.5%, 50%, and 25% of the evaluated cross combinations displayed positive ID for plant height, number of stems, and incidence of late blight disease per plot, respectively.

3.4. Correlation

The correlation analysis revealed highly significant and positive associations ($p < 0.01$) among several yield-attributing traits, suggesting that improvement in one trait may positively influence others and ultimately enhance overall tuber yield. Specifically, tuber yield per plot exhibited highly significant and positive correlations with marketable tubers per plot ($r = 0.86^{**}$), total tubers per plot ($r = 0.69^{**}$), weight of marketable tubers per plot ($r = 0.79^{**}$), and total tuber weight per plot ($r = 1.00^{**}$). These strong associations indicate that these traits play a pivotal role in determining final yield and can serve as reliable selection criteria for yield improvement in potato breeding programs (Table 7). Conversely, tuber yield per plot showed a positive but non-significant correlation with days to emergence ($r = 0.15^{NS}$), plant height ($r = 0.15^{NS}$), and number of stems per plant ($r = 0.25^{NS}$), suggesting that these growth parameters have a limited direct effect on yield under the current environmental conditions.

4. Discussion

The assessment of heterosis and inbreeding depression forms the cornerstone of modern plant breeding programs, as these genetic phenomena influence hybrid performance, adaptability, and the sustainability of improved varieties across various locations. The success of potato breeding largely depends on developing cultivars with high yield potential, strong resistance to disease, and favorable yield attributing characters, which collectively ensure breeding efficiency, production stability, and sustainable food security in changing agro-climatic conditions [1]. The broad range in mean trait values indicates that these hybrids maintained consistent and enhanced performance, particularly with respect to tuber yield, disease resistance, and yield-contributing characteristics as highlighted by [21, 26, 27]. Such pronounced variability underscores the presence of exploitable genetic diversity, which is essential for identifying superior genotypes and advancing selection efficiency in future potato breeding programs. These findings align with recent studies by [28, 29], who reported that TPS-derived potato hybrids possess wide genetic variation and exhibit strong adaptability across contrasting growing environments, thereby offering substantial potential for yield improvement, sustainable production and improves both market value and resilience of potato cultivation systems [30, 31].

In the current study, potato hybrids expressed a broad range of heterotic effects for relative heterosis (1.07%–64.10%), heterobeltiosis (5.32%–54.58%), and standard heterosis (–8.19%–48.64%) across studied characters. These results reaffirm the importance of heterosis breeding as a key strategy for improving traits governed predominantly by dominant gene action, also explained by [32-35] where they also documented significant positive heterotic effects in potato hybrids for yield and related traits, highlighting the potential of heterosis-based selection in enhancing productivity. Early-emerging hybrids tend to utilize available soil moisture and nutrients more efficiently, resulting in vigorous initial growth and better tuber bulking before the onset of adverse weather conditions. Similarly, hybrids expressing negative heterosis for late blight incidence demonstrate enhanced genetic resistance and reduced pathogen susceptibility, both of which are essential for sustainable potato production under climate-vulnerable environments [36]. The presence of negative heterosis for majority of cross combinations emphasized the role of heterosis in conferring disease resistance and early crop vigor in potato [37]. Negative heterotic expression for late blight resistance has also been linked to the expression of multiple resistance (R) genes and quantitative trait loci (QTLs) associated with pathogen recognition and defense activation. Therefore, the current results underscore the importance of heterotic breeding strategies for developing early-maturing, disease-resistant, and high-yielding potato cultivars suitable for high-altitude and stress-prone environments such as Northern Pakistan.

In potato breeding, positive heterotic expression for vegetative growth traits particularly plant height, stems number, and leaf area per plant is highly desirable, as it promotes vigorous canopy development, enhances photosynthetic efficiency, and improves nutrient assimilation, all of which are crucial determinants of biomass accumulation and overall yield performance [38]. Meanwhile negative heterotic responses may result from genetic incompatibility, allelic imbalance, or environmental interactions that restrict vegetative growth [36]. Overall, the results emphasize that specific hybrid combinations express strong positive heterosis for key growth traits, offering valuable genetic potential for developing vigorous, high-yielding, and stress-resilient cultivars. These findings align with the findings and recent reports highlighted by [39, 40]. Conversely, inbreeding depression remains a major obstacle in potato improvement. It reduces vigor, fertility, and overall plant fitness through the expression of deleterious recessive alleles accumulated over generations of selfing [14, 41]. The present study observed varying levels of negative inbreeding depression across several yield-related traits, indicating a decline in performance after selfing, consistent with earlier findings by [42].

The observed positive heterosis across yield-related traits indicates a strong potential for exploiting hybrid vigor in potato breeding through true potato seed (TPS) based hybridization. Similarly, the researchers also emphasized that hybrids with consistently positive heterotic expression for marketable yield components often perform well across diverse environments [43, 44]. These results further underscore the genetic complementarily between the parental lines used, particularly in Sarpomira × Roko, which appears to possess a high degree of additive and non-additive gene action, making it a promising candidate for the development of high-yielding and disease-resilient potato cultivars. It was noted that diploid true potato seed (TPS) breeding can facilitate more predictable inheritance patterns and efficient selection for complex traits such as yield and disease resistance [45]. Recent progress in diploid hybrid breeding has further confirmed its potential to deliver genetically uniform, disease-resistant, and high-yielding varieties adaptable to diverse agro-ecological conditions [46]. Positive heterosis for number and weight of marketable tubers per plot, total number of tubers, total tuber weight, and tuber yield per plot is equally important, as these parameters are closely linked to yield sustainability, nutritional improvement, and economic viability of potato production [47-49].

The predominance of negative ID values across yield-related traits suggests a substantial loss of hybrid vigor in the F_2 generation, a phenomenon commonly reported in self or segregating generations of cross-pollinated crops like potato [44]. This reduction can be attributed to the breakdown of dominant and epistatic gene interactions that previously contributed to heterotic performance in the F_1 generation [38, 50]. Likewise, it was observed that significant inbreeding depression for tuber yield and marketable tuber weight in TPS-derived potato hybrids, emphasizing the importance of hybrid stability and recurrent selection in early generations [51]. Positive inbreeding depression for growth-related parameters may indicate over-dominance or dominant gene effects, which tend to stabilize performance across generations [43]. Overall, these findings underscore the importance of maintaining heterozygosity and exploiting hybrid vigor through recurrent selection, poly-crossing, or controlled heterotic grouping in potato breeding programs. The hybrid combinations exhibiting moderate to low inbreeding depression, particularly Sarpomira × Roko, appear promising for further advancement, as they demonstrate resilience against the loss of vigor and yield potential observed in other crosses.

The negative and non significant correlation of tuber yield per plot with disease incidence ($r = -0.31^{NS}$) and leaf area per plot ($r = -0.01^{NS}$) implies that genotypes with better disease resistance tend to produce higher yields, a relationship consistent with findings by [49, 52], who reported that minimizing disease pressure through resistant hybrids directly supports tuber yield stability. The combination of substantial genetic variability, broad diversity, and positive heterotic effects, coupled with manageable levels of inbreeding depression, points toward the predominance of additive and partial dominance gene action in controlling yield and associated traits. The superior performance of hybrids such as Sarpomira × Roko and Kuroda × Burna underscores their potential as promising parental lines for future hybridization and selection programs. Furthermore, the identification of Battakundi as an optimal testing site highlights the importance of evaluating TPS hybrids across environmentally diverse regions, ensuring stable yield performance and adaptability. Overall, the findings demonstrate that heterosis breeding, supported by careful management of inbreeding depression and strategic use of genetically diverse parental lines, can play a transformative role in developing resilient, high-yielding potato hybrids. This approach will contribute substantially to food security, climate resilience, and sustainable potato production in Northern Pakistan and other high-altitude regions with similar agro-ecological characteristics.

5. Conclusions

The study revealed substantial heritable variations, broad genetic diversity, and pronounced heterosis with negligible inbreeding depression among the evaluated potato hybrids, highlighting strong potential for future breeding advancement. The crosses like Sarpomira × Roko and Kuroda × Burna emerged as the most promising candidates for commercial release. Battakundi proved to be an optimal environment for potato cultivation and continued breeding efforts. Adopting the true potato seed (TPS) approach in Pakistan can enhance yield, resilience, and quality traits, promoting sustainable potato production, strengthening food security, and supporting economic growth in the region.

6. Key Findings

Among cross combinations, Sarpomira × Roko and Kuroda × Burna proved to be the most promising hybrids, owing to their superior production and positive heterotic response for yield components, and favorable negative heterosis for days to maturity. Battakundi has been identified as a highly suitable environment for potato cultivation and declared as a potential hotspot for future breeding initiatives.

Abbreviations

TPS	True Potato Seed
Het.	Heterosis
ID	Inbreeding Depression
MP	Mid Parent Heterosis
RH	Relative Heterosis
BP	Better Parent Heterosis
HB	Heterobeltiosis
SH	Standard Heterosis
FAO	Food and Agriculture Organization
DAP	Diammonium Phosphate
SOP	Sulphate of Potash
ANOVA	Analysis of Variance
LSD	Least Significant Differences
P	Probability
DE	Days to Emergence
PH	Plant Height
NOS/P	Number of Stem per Plot
LA/Pl.	Leaf Area per Plant
NMT/P	Number of Marketable Tubers per Plot
NTT/P	Number of Total Tubers per Plot
WMT/P	Weight of Marketable Tubers per Plot
TTW/P	Total Tubers Weight per Plot
TTY/P	Tuber Yield per Plot
LBD/P	Late Blight Disease per Plot
m	Meter
cm	Centimeter
mm	Millimeter
t ha^{-1}	Tons per Hectare

Acknowledgments

Authors acknowledged Hazara Agriculture Research Station Abbottabad, Seed Potato Research and Multiplication Farm Battakundi, and the University of Haripur Khyber Pakhtunkhwa Pakistan for their support and cooperation throughout the potato breeding program and research study.

Author Contributions

Bilal Ahmed Khan: Conceptualization, Writing – original draft

Naushad Ali: Project Administration, Supervision, Writing – review & editing

Sardar Ali: Investigation

Muhammad Tehseen Zaheer Tanoli: Methodology

Maria Saleem: Data curation

Hassan Tariq: Formal Analysis, Software

Muhammad Kifayatullah: Resources

Conflicts of Interest

The authors declare no conflicts of interest.

Appendix

Table 1. Pedigrees and history of parental cultivars used for hybridization.

No.	Parental Cultivars	Pedigree	Origin	Year of Release
1	Sarpomira	76.PO.12.14.268 × D187	Hungary	2003

No.	Parental Cultivars	Pedigree	Origin	Year of Release
2	Roko	ALWARA × MA 81-536	Austria	1998
3	Cardinal	TULNER/DEVRIES54-30-8 × SVP55-89	Holland	1972
4	Kuroda	AR 76-199-3 × KONST 80-1407	Holland	1998
5	Burna	KARDAL × SPOLIA	Germany	2007

Table 2. Henfling modified disease estimation scale to observe incidence to late blight of F_1 potato hybrids.

Grade	% Incidence	Nature of Infection (Level of Resistance/ Susceptibility)
0	0.0%	No disease
1	10%	Small lesions on the inoculated point with the lesion area less than 10% of the whole leaflet
3	10.0 – 20.0%	Lesions area between 10% and 20% of the whole leaflet
5	20% – 30%	Lesion area between 20% and 30% of the whole leaflet
7	30% – 60%	Lesion area between 30% and 60%
9	Over 60%	Lesion area over 60% of the whole leaflet

Table 3. Mean square and coefficient of variations of different traits in studied environments.

Sites	Source	DE	PH (cm)	NOS/Pl.	LA/Pl. (cm^2)	NMT/P	NTT/P	WMT/P	TTW/P (Kg)	TY/P (ton ha^{-1})	LBD/P (%)
Abbottabad	Replication	3.410	9.633	0.5384	6.0307	16.1795	1207.7	2.04077	16.1403	79.705	0.0215
	Treatment	19.52**	203.9**	1.50*	34.37**	76.78**	1050.3**	2.1539*	6.882*	33.98*	0.085**
	Error	1.438	7.867	0.538	5.1850	10.6517	308.66	0.72327	2.3572	11.640	0.01126
	CV (%)	3.81	6.52	18.34	8.04	16.53	9.86	23.57	12.10	12.10	43.11
Battakundi	Replication	6.256	38.844	0.076	0.441	82.231	220.49	2.611	8.083	39.917	0.020
	Treatment	55.500**	178.349**	2.158**	65.32**4	130.936**	3501.74**	2.875**	18.490**	91.311**	0.077**
	Error	4.089	15.645	0.465	10.820	13.397	464.82	0.480	2.533	12.513	0.017
	CV (%)	5.67	8.01	14.79	10.54	12.90	9.27	14.86	9.55	9.55	44.45

*, **: Indicates significant at 5% and 1% level of probability, respectively, CV = Coefficient of variation; DE = Days to emergence; PH = Plant height; NOS/Pl. = Number of stems plant^{-1}; LA/Pl. = Leaf area plant^{-1}; NMT/P = Number of marketable tubers plot^{-1}; NTT/P = Number of total tubers plot^{-1}; MTW/P = Weight of marketable tubers plot^{-1}; TTW/P = Total tubers weight plot^{-1}; TY/P = Tubers yield plot^{-1}; LBD/P = Late blight disease plot^{-1}.

Table 4. Range observed for relative heterosis, heterobeltiosis and standard heterosis of various traits in F_1 potato hybrids.

Parameters	Relative Heterosis% (RH)		No. of Significant RH	Hetero-beltiosis% HB)		No. of Significant HB	Standard Heterosis% (SH)		No. of Significant SH
	Min.	Max.		Min.	Max.		Min.	Max.	
DE	-0.57	-1.58	0	5.32	30.08	0	-4.10	-11.76	2

Parameters	**Relative Heterosis% (RH)**		**No. of Significant RH**	**Hetero-beltiosis% HB)**		**No. of Significant HB**	**Standard Heterosis% (SH)**		**No. of Significant SH**
	Min.	**Max.**		**Min.**	**Max.**		**Min.**	**Max.**	
PH (cm)	0.00	35.94	0	7.20	30.98	3	2.86	20.04	2
NOS /Pl.	23.80	52.38	0	17.11	44.14	0	17.11	44.14	6
LA /Pl. (cm^2)	0.91	13.88	0	4.03	4.09	0	0.13	22.05	7
NMT /P	4.27	64.10	4	3.55	54.58	5	5.72	40.96	2
NTT /P	4.08	15.68	8	0.86	13.87	8	5.75	23.41	5
WMT /P (Kg)	1.07	63.76	1	13.97	44.87	0	1.80	48.64	4
TTW /P (Kg)	1.11	19.21	1	11.11	14.25	4	3.70	34.75	5
TY /P (Tonsha^{-1})	0.98	20.03	6	10.97	14.12	4	4.10	35.27	6
LBD /P (%)	-44.44	-72.7	0	-48.71	-61.53	1	-16.66	-83.33	0

DE = Days to emergence, PH = Plant height, NOS/Pl. = Number of stems plant^{-1}, LA/Pl. = Leaf area plant^{-1}, NMT/P = Number of marketable tubers plot^{-1}, NTT/P = Number of total tubers plot^{-1}, WMT/P = Weight of marketable tubers plot^{-1}, TTW/P = Total tubers weight plot^{-1}, TY/P = Tubers yield plot^{-1}, LBD/P = Late blight disease plot^{-1}.

Table 5. Relative heterosis (RH) and heterobeltiosis (HB) of various traits in F_1 potato hybrids.

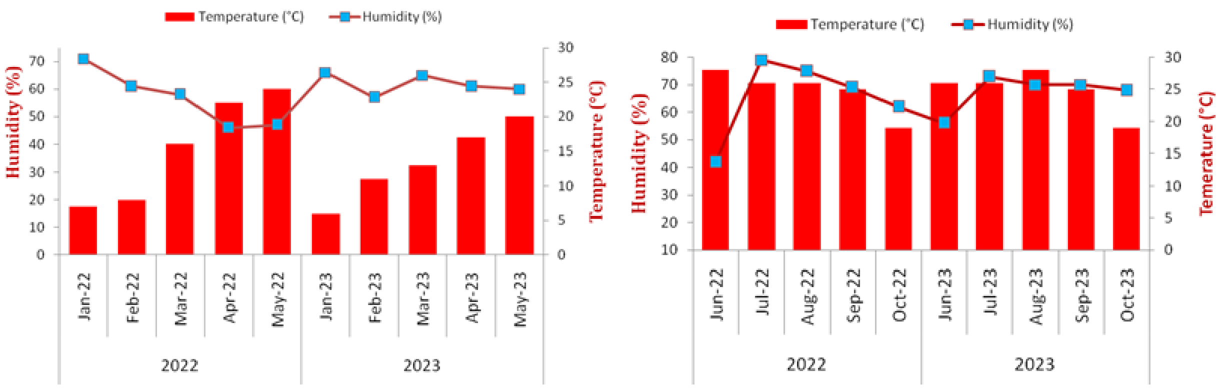
F1 Hybrids	**Heterosis (%)**	**DE**	**PH (cm)**	**NOS/Pl**	**LA/Pl (cm^2)**	**NMT/P**	**NTT/P**	**WMT/P (Kg)**	**TTW/P (Kg)**	**TY/P (Ton ha^{-1})**	**LB/P (%)**
K × B	RH (%)	3.85	0	-9.09	-18.62	53.08	10.65*	63.76	19.21	20.03*	-60
	HB (%)	6.89	-8.21	-11.76	-26.84	40.58	0.86*	44.87	11.20*	10.97*	-48.71
C × B	RH (%)	4.71	26.77	31.31	-1.91	35.59	-16.10*	33.33	12.32*	11.68*	-72.72
	HB (%)	6.00	11.89	27.45	-8.67	16.50*	-20.86*	15.31	11.38*	11.24*	-61.53
K × R	RH (%)	2.13	30.19	41.41	2.58	30.71	-4.26*	35.89	-1.72	-1.42**	-58.62
	HB (%)	5.69	24.34**	37.25	-0.79	18.7*	-9.09*	13.97	-11.62	-11.73	-50
S × R	RH (%)	-1.58	29.56	33.33	13.88*	64.10	15.68*	48.64	19.66	20.96*	-64.70
	HB (%)	8.01	19.44	26.12	4.03	54.58	2.48*	30.95	14.25*	14.12*	0
C × R	RH (%)	11.11	21.33	31.31	-8.38	4.27*	10.57*	5.88	14.66	14.98*	-54.83
	HB (%)	11.89	20.96**	27.45	-14.64	-1.29*	13.87*	-2.70	11.11*	10.98*	-49.27
S × K	RH (%)	-0.51	23.51	23.80	3.71	-23.23**	-7.34*	12.90	-10.83	-10.94	-66.66
	HB (%)	5.32	19.01	17.11	-2.23	-38.51	-18.10*	-16.66	-22.46	-22.54	0
R × S	RH (%)	18.51	16.29	52.38	0.91	9.40*	-5.24*	6.30	-1.99	-1.73	-44.44
	HB (%)	30.08	7.20	44.14	-7.81	3.55*	-12.17*	-6.34	-4.83	-4.93	0
K × S	RH (%)	4.61	35.94	23.80	10.43	15.15*	4.08*	1.07**	1.11	0.98**	66.66
	HB (%)	10.74	30.98**	17.11	4.09	-7.76*	-8.05*	-25.39	-12.07	-12.17	0

K × B = Kuroda × Burna, C × B = Cardinal × Burna, K × R = Kuroda × Roko, S × R = Sarpomira × Roko, C × R = Cardinal × Roko, S × K = Sarpomira × Kuroda, R × S = Roko × Sarpomira, K × S = Kuroda × Sarpomira.

Table 6. Standard heterosis (SH) and inbreeding depression (ID) of various traits in F_1 potato hybrids.

F1 Hybrids	Heterosis (%)	DE	PH (cm)	NOS/Pl	LA/Pl (cm^2)	NMT/P	NTT/P	WMT/P (Kg)	TTW/P (Kg)	TY/P (Tons ha^{-1})	LB/P (%)
K × B	SH (%)	-8.82	20.04*	-18.91	-19.82*	-8.95*	13.29*	1.80*	13.10*	13.54	-66.66
	ID (%)	-26.87	2.84	-55.55	-6.60	-43.54	-38.35	-31.85	-45.09	-45.09	-25.05
C × B	SH (%)	-11.76	-2.52	17.11*	-7.46	5.72	-11.11	15.31*	14.24*	14.68	-75
	ID (%)	-31.1	-17.83	-23.07	-11.79	-59.72	-77.23	-42.18	-48.37	-48.38	-133.3
K × R	SH (%)	-10.78	-9.47	26.12*	16.39*	-11.89	-6.54	-4.50	-2.56	-2.18	-50
	ID (%)	-32.95	-35.54	7.14	-6.82	-51.66	-66.03	-38.68	-63.74	-63.75	-33.35
S × R	SH (%)	-8.82	-6.20	26.12*	22.05*	40.96*	23.41*	48.64**	34.75**	35.27*	-75
	ID (%)	-19.35	-15.04	-28.57	-13.81	11.45	-34.08	18.78	-19.66	-19.66	-133.3
C × R	SH (%)	-6.86	-19.35	17.11*	0.13*	-10.42	17.06*	-2.70	22.50*	22.98*	-41.66
	ID (%)	-19.99	-43.70	0	-7.17	-19.67	-39.66	-32.40	-32.56	-32.55	-71.45
S × K	SH (%)	-4.90	-6.54	17.11*	7.14*	-44.19	-1.38	-5.40	-8.54	-8.19	-83.33
	ID (%)	-7.19	1.38	7.69	-30.80	-223.6	-24.54	-86.66	-61.68	--61.67	-349.7
R × S	SH (%)	9.80**	-15.81	44.14	8.15*	-6.02	5.75*	6.30*	12.25*	12.68*	-16.66
	ID (%)	-9.82	-27.28	-18.75	9.61	-14.06	-21.19	-4.23	-28.43	-28.42	19.98
K × S	SH (%)	0	2.86*	17.11*	14.08*	-16.29	10.71*	-15.31	3.70*	4.10*	-16.66
	ID (%)	-15.67	6.56	7.69	-0.81	-22.80	-10.75	-42.55	-36.26	-36.26	19.98

K × B = Kuroda × Burna, C × B = Cardinal × Burna, K × R = Kuroda × Roko, S × R = Sarpomira × Roko, C × R = Cardinal × Roko, S × K = Sarpomira × Kuroda, R × S = Roko × Sarpomira, K × S = Kuroda × Sarpomira.



(A) Abbottabad (B) Battakundi

Figure 1. Average Mean temperature (°C) and humidity (%) for different environments. (Source: www.climate-data.org).

Table 7. Correlation of tuber yield and yield attributing traits.

Traits	DE	PH (cm)	NOS/Pl	LA/Pl (cm^2)	NMT/P	NTT/P	WMT/P (Kg)	TTW/P (Kg)	TY/P (Tons ha^{-1})
PH	0.18^{NS}								

Traits	**DE**	**PH (cm)**	**NOS/Pl**	**LA/Pl (cm^2)**	**NMT/P**	**NTT/P**	**WMT/P (Kg)**	**TTW/P (Kg)**	**TY/P (Tons ha^{-1})**
NOS/Pl	0.60*	0.06^{NS}							
LA/Pl	0.11^{NS}	-0.40^{NS}	0.48^{NS}						
NMT/P	0.07^{NS}	0.20^{NS}	0.39^{NS}	0.18^{NS}					
NTT/P	0.29^{NS}	0.18^{NS}	-0.01^{NS}	-0.08^{NS}	0.41^{NS}				
WMT/P	0.20^{NS}	0.24^{NS}	0.45^{NS}	0.11^{NS}	0.89**	0.41^{NS}			
TTW/P	0.15^{NS}	0.15^{NS}	0.25^{NS}	-0.01^{NS}	0.86**	0.69**	0.79**		
TY/P	0.15^{NS}	0.15^{NS}	0.25^{NS}	-0.01^{NS}	0.86**	0.69**	0.79**	1.00**	
LBD/P	-0.29^{NS}	-0.54*	-0.26^{NS}	0.30^{NS}	-0.28^{NS}	-0.29^{NS}	-0.54*	-0.31^{NS}	-0.31^{NS}

** = P< 0.01; * = P< 0.05; and ns = P> 0.05; DE = Days to emergence, PH = Plant height; NOS/Pl. = Number of stems plant^{-1}, LA/Pl. = Leaf area plant^{-1}, NMT/P = Number of marketable tubers plot^{-1}, NTT/P = Number of total tubers plot^{-1}, WMT/P = Weight of marketable tubers plot^{-1}, TTW/P = Total tubers weight plot^{-1}, TY/P = Tubers yield plot^{-1}, LBD/P = Late blight disease plot^{-1}.

References

[1] Khan BA, Ali N, Ali S, ASLAM S, Malik A. A (2025). Heterosis and inbreeding depression in potato (*Solanumtuberosum* L.) hybrids developed through true potato seed in northern Pakistan. *SABRAO J. Breed. Genet.* 57(3): 977 – 988.

[2] Luitel BP, Paneru RB, Sharma PN, Gauchan DP (2020). Nutritional composition and importance of potato in human diet: A review. *J. Food Sci. Nut.* 8(4): 112–120.

[3] FAO (2024). Food and Agriculture Organization of the United Nations. Record Global Potato Crop: Analysis and Trends. Statistical Database.

[4] PCCMD (2024). Punjab Crop Reporting Service. Food and Nutrition Facts Report. Punjab Food Authority, Government of the Punjab.

[5] Zhang Y, Liu H, Wang J, Chen F (2023). Advances in understanding the genetic and molecular basis of heterosis in crop plants. *Frontiers Plant Sci.* 14: 1123456.

[6] Li J, Zhang Y, Hu X (2022). Analysis of heterosis and genetic basis of yield traits in potato hybrids using molecular markers. *Plant Breed.* 141(2): 232–243.

[7] Singh P, Sharma R, Kumar D (2024). Exploiting heterosis for yield and quality improvement in potato (*Solanumtuberosum* L.). *J. Crop Improvement.* 38(2): 155–170.

[8] Mohammad T, Khan S, Rahman H (2013). Heterosis and combining ability analysis for yield and yield components in potato. *Pakistan J. Bot.* 45(5): 1589–1595.

[9] Ali M, Jatoi SA, Khan MA (2023). Genetic basis and expression of heterosis in potato: Implications for hybrid breeding. *Plant Breed.* 142(3): 213–225.

[10] Chen L, Wang S, Zhao Y (2024). Genetic consequences of inbreeding and its mitigation in crop plants. *Frontiers in Genet.* 15: 1324567.

[11] Prajapati R, Singh D, Patel P (2023). Understanding inbreeding depression and its impact on crop improvement. *The Plant Genome.* 16(1): e20289.

[12] Zhang C, Hoopes G, Jansky SH (2020). Inbreeding depression and its genetic basis in clonally propagated potato. *Theor. Appl. Genet.* 133(3): 873–888.

[13] Jansky SH, Bethke PC, Endelman JB (2023). Challenges and progress in diploid potato breeding for hybrid development. *Crop Sci.* 63(2): 612–625.

[14] Labroo MR, Ramasamy S, Jansky SH (2023). Diploid breeding and the genetic potential of true potato seed systems. *Plants.* 12(9): 1857.

[15] Jansky SH, Douches DS (2024). Transitioning to diploid hybrid breeding in potato: Opportunities and challenges. *Plant Breed. Reviews.* 48: 201–230.

[16] Hussain A, Shah Z, Rehman M (2024). Agro-ecological potential and yield dynamics of potato under different climatic zones of Pakistan. *Frontiers Agron.* 6: 1342851.

[17] Rashid M, Bukhari S, Farooq M (2023). Seed potato production constraints and opportunities in Pakistan: A review. *J. Plant Breed. Genet.* 55(2): 145–158.

[18] Majeed A, Muhammad Z (2018). Current status and prospects of potato production in Pakistan. *Pakistan J. Agric. Sci.* 55(1): 13–21.

[19] Ali F, Jatoi SA, Khan M (2024). Evaluating the potential of true potato seed (TPS) for sustainable potato production in Pakistan. *Int. J. Agric. Biol.* 32(1): 88–96.

[20] Majeed A, Khan MA, Shah S (2017). Constraints in seed potato production and certification system in Pakistan. *Sarhad J. Agric.* 33(3): 412–419.

[21] Asnake W, Tesfaye T, Mekonnen D (2023). Assessment of heterosis and combining ability in true potato seed (TPS)-derived hybrids under contrasting environments. *J. Plant Breed. Genet.* 41(2): 87–103.

[22] Mohsin AU, Khan MA, Javed H, Shah SA (2016). Screening of potato germplasm against late blight disease under natural field conditions. *Pakistan J. Phytopath.* 28(1): 53–59.

[23] Tessema L, Muhammad W, Abebe T (2020). Evaluation of potato (*Solanumtuberosum* L.) varieties for yield and some agronomic traits. *Open Agric.* 5: 63–74.

[24] Khaliqi A, Abdullah M, Khan M (2021). Genetic variability, heritability and genetic advance for yield and related traits in Bambara groundnut. *Agronomy.* 11(8): 1634.

[25] Sharma R, Kumar D, Singh P (2023). Correlation and path coefficient analysis for yield and its related traits in potato (*Solanumtuberosum* L.) genotypes. *Int. J. Current Microbiol. Appl. Sci.* 12(5): 210–219.

[26] Subedi A, Pandey B, Gautam D (2021). Genetic variability and association studies in potato genotypes under field conditions. *Nepal J. Agric. Sci.* 20(1): 15–27.

[27] Zeleke H, Tsegaye D, Alemu B (2021). Evaluation of heterosis and heritability in true potato seed-derived hybrids under different agro-ecologies. *African Crop Sci. J.* 29(4): 457–471.

[28] Tessema L, Bizuayehu D, Fekadu H (2023). Evaluation of true potato seed (TPS) hybrids for yield stability and adaptability under diverse agro-ecological zones. *American J. Plant Breed.* 17(2): 89–101.

[29] Zhang Y, Li X, Wang J (2022). Genetic variability and agronomic performance of TPS-derived potato genotypes across multiple environments. *Frontiers Plant Sci.* 13: 1002456.

[30] Bonierbale M, Amoros W, Forbes G (2020). Genetic improvement of potato for biotic and a-biotic stress tolerance. *Pot. Res.* 63(4): 395–418.

[31] Ene CO, Ogbonna PE, Agu, KC (2016). Genetic variability and heritability of yield and yield components of potato genotypes. *African J. Agric. Res.* 11(30): 2749–2757.

[32] Kumar R, Patel D, Singh P (2023). Assessment of heterosis and combining ability in potato (*Solanumtuberosum* L.) for yield and its components. *Euphytica.* 219(4): 1–14.

[33] Sharma N, Meena RK, Kumar S (2022). Estimation of heterosis for yield and yield-contributing traits in potato hybrids under temperate conditions. *J. Crop Improvement.* 36(5): 687–702.

[34] Li X, Zhao Y, Wang Y, Qian W (2022). Heterosis in crops: Genetic mechanisms and applications in hybrid breeding. *Plants.* 11(9): 1205.

[35] Ortiz R, Mihovilovich E, Crossa J (2022). Heterosis and genetic gain in potato: New insights from hybrid breeding programs. *Theo. Appl. Genet.* 135(12): 3865–3878.

[36] Campos H, Ortiz O, Vosman B (2022). Advances in potato breeding for disease resistance and climate resilience. *Theo. Appl. Genet.* 135(5): 1527–1543.

[37] Sharma P, Kaur G, Pandey A (2023). Heterosis and combining ability for vegetative vigor and yield traits in potato (*Solanumtuberosum* L.). *Plant Breed. Biotechnol.* 11(2): 64–76.

[38] Singh S, Meena RK, Chauhan S (2023). Exploitation of heterosis for yield and yield components in potato under temperate conditions. *Pot. Res.* 66(3): 275–290.

[39] Karki B, Paudel R, Neupane D (2024). Heterotic response and growth performance of potato hybrids under highland agro-ecological conditions. *Euphytica.* 220(1): 12.

[40] Sharma P, Kaur G, Pandey A (2023). Heterosis and combining ability for yield and earliness in potato (*Solanumtuberosum* L.). *Plant Breed. Biotechnol.* 11(1): 24–35.

[41] Gopal J (2014). Breeding behavior and genetic improvement of potato (*Solanumtuberosum*L.): Achievements and prospects. *Euphytica.* 200(1): 1–19.

[42] Almekinders CJM, Struik PC, Malagamba P (2009). The role of informal seed systems in the diffusion of improved potato varieties: A case study in the Andes. *Agric. Sys.* 101(3): 190–201.

[43] Wang L, Zhang H, Zhou X (2023). Quantitative genetic analysis of heterosis and inbreeding depression in potato under different environments. *Euphytica.* 219(5): 112–128.

[44] Gebremedhin W, Tadesse M, Alemu B (2024). Heterotic expression and yield performance of potato hybrids under varying agro-ecological zones. *J. Crop Improvement.* 38(2): 145–162.

[45] Lindhout P, de Vries M, terMaat M, Suherman R (2017). Hybrid true potato seed as a novel breeding approach for global potato cultivation. *Plant Biotechnol. J.* 15(4): 564–574.

[46] Zhang W, Chen Y, Li P (2024). Genetic mapping and trait dissection in diploid potato hybrids reveal potential for hybrid breeding. *Hort. Res.* 11: 182.

[47] Mekonnen D, Tesfaye T, Bekele H (2023). Genetic variability and trait association analysis in potato (*Solanumtuberosum* L.) hybrids under field conditions. *African J. Agric. Res.* 19(8): 432–444.

[48] Sharma V, Patel D, Joshi R (2024). Heterosis and combining ability studies for yield and its components in potato (*Solanumtuberosum* L.). *Int. J. Vegetable Sci.* 30(1): 88–102.

[49] Singh R, Verma, A, Patel M (2023). Estimation of heterosis and combining ability for growth and yield-related traits in potato hybrids. *Euphytica.* 219(3): 87.

[50] Mekonnen D, Tesfaye T (2023). Evaluation of yield and marketable tuber traits in true potato seed (TPS) derived hybrids. *African J. Agric. Res.* 19(8): 432–444.

[51] Sharma V, Patel D, Joshi R (2024). Inbreeding depression and genetic stability analysis in true potato seed-derived hybrids. *Int. J. Vegetable Sci.* 30(1): 88–102.

[52] Prajapati P, Singh N, Kumar R (2024). Genetic variability, heterosis, and correlation analysis in potato (*Solanumtuberosum* L.) hybrids derived from true potato seed. *Plant Breed. Biotechnol.* 14(1): 23–39.