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HETEROSIS AND INBREEDING DEPRESSION IN POTATO (*SOLANUM TUBEROSUM* L.) HYBRIDS DEVELOPED THROUGH TRUE POTATO SEED IN NORTHERN PAKISTAN

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SUMMARY

Heterosis and inbreeding depression are key considerations in potato breeding, particularly in regions with limited resources. Heterosis (F_1) and inbreeding depression (F_2) investigations ensued in 2022–2024 in eight different potato (*Solanum tuberosum* L.) hybrids generated from true potato seeds in Abbottabad and Battakundi, Pakistan. A randomized complete block design experiment transpired with three replications. The analysis of variance revealed significant differences among the potato hybrids for all traits, except the tuber germination percentage, signifying a substantial level of genetic variability. The Cardinal × Roko hybrid has demonstrated exceptional hybrid vigor for the number of medium tubers plot^{-1}, with high estimates of relative heterosis (61.78%), heterobeltiosis (61.78%), and standard heterosis (86.01%). The negative inbreeding depression observed for all studied traits, ranging from –0.364% to –187.67%, indicates inbreeding has significantly impacted the performance of hybrids. The noted positive correlations among potato traits provide valuable insights for breeding programs. Results concluded the cross combinations, Cardinal × Roko and Kuroda × Burna, exhibited significant and high heterotic values for the number and weight of medium tubers plot^{-1}, while desirable negative heterosis for days to maturity. It confirms these crosses have strong potential for release as new potato hybrids. Further evaluation of these hybrids in future breeding strategies can probe ultimate potential for resilience, yield, and other important traits.

Keywords: Potato, true potato seed, heterosis, inbreeding depression, Cardinal × Roko, Kuroda × Burna

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Key findings: Given the heterotic effects, the hybrids' cross combinations demonstrated superior mean performance for the evaluated traits compared with their parental cultivars. Cardinal × Roko and Kuroda × Burna were the best crosses due to the high number and weight of medium tubers plot^{-1}, positive heterosis, and desirable negative heterosis for days to maturity. Additionally, Battakundi has become a valid ideal environment for potato cultivation and future breeding programs.

INTRODUCTION

The challenge of meeting the food requirements of a rapidly increasing global population is one of the most burning issues faced by the world today. Potatoes (*Solanum tuberosum* L.) indeed play a critical role in global food security due to their versatility, nutritional values, and adaptability. They are rich in essential nutrients, particularly vitamin C, and various other micronutrients, such as, vitamin B6, potassium, folate, and iron (Luitel *et al*., 2020). It is an annual crop in the genus *Solanum* of the family Solanaceae (Brittanica, 2023). Potato production is a complex and dynamic process influenced by various factors, including variety selection, climate, soil conditions, and agricultural practices. According to FAO data, global potato production reached 376 million tons harvested from an area of approximately 18.1 million hectares (FAOSTAT, 2023). In Pakistan, the area under potato cultivation was 341,000 hectares, with an average production of 8,319,000 tons (PBS, 2022–2023).

Heterosis (hybrid vigor) refers to the phenomenon where F_1 hybrids (progeny from a cross between two different parental cultivars) show superior performance compared with their parents for different traits like yield, growth, and fertility (Li *et al.,* 2022). This is a powerful and reliable tool in potato breeding, enabling breeders to make informed decisions and optimize traits effectively. Potato breeders can develop new varieties and hybrids that leverage positive heterosis to achieve higher tuber yields while also utilizing negative heterosis to enhance disease resistance and promote early maturity. Mohammad *et al.* (2013) emphasized the significance of heterosis in potato breeding, identifying it as a key factor in developing economically viable and high-quality potato hybrids. Three different types of heterosis are relative heterosis (mid-parent), heterobeltiosis (better parent), and standard heterosis (standard parent), which can result from various genetic mechanisms, including partial to complete dominance, overdominance, epistasis, or their combined effects (Khan *et al.,* 2017). Understanding these types is essential for maximizing hybrid vigor in potato breeding programs.

Inbreeding depression refers to the decrease or loss in yield, vigor, fertility, and overall production due to inbreeding (Prajapati *et al*., 2023). This decline occurs as a result of reduced genetic diversity, leading to the expression of harmful recessive alleles negatively affecting the performance of individuals within a population. Zhang *et al.* (2019) discussed the challenges of inbreeding depression in clonally propagated crops, such as potatoes. In these crops, inbreeding depression can be particularly severe because they are propagated vegetatively rather than through sexual reproduction. This method limits genetic diversity, increasing the likelihood of expressing harmful recessive alleles and negatively impacting yield and vigor. Additionally, lethal mutations can be a significant factor contributing to inbreeding depression in potatoes (Zhang *et al*., 2019). Therefore, both heterosis and inbreeding depression are critical considerations in potato breeding programs, especially in regions with limited modern facilities like Northern Pakistan, where potato growers often rely on traditional farming practices and old potato varieties for domestic use. Addressing inbreeding depression and enhancing potato breeding programs requires essential focus on harnessing genetic variability and utilizing true potato seed (TPS), also known as the botanical seed. This approach can help improve genetic diversity and overall crop performance, leading to more resilient and productive potato varieties.

Generally, three potato crop growing seasons happen in Pakistan, namely, spring, summer, and autumn. These crop cultivations proceed across various environmental conditions, ranging from plains to hilly areas, allowing for diverse production practices and adaptation to regional climates (Majeed and Muhammad, 2018). Despite this, Pakistan faces challenges in achieving higher potato production due to several factors, including the lack of true-to-type, disease-free certified seeds, the absence of improved potato varieties, difficulties in identifying suitable growing conditions, and inadequate crop management practices (Majeed *et al*., 2017a). Addressing these issues is essential for enhancing potato yield and ensuring a more resilient production system. True potato seed (TPS) is a promising breeding approach of significant value, particularly in high-altitude potato production regions like Northern Pakistan. True potato seed (TPS) offers advantages, such as improved disease resistance, enhanced genetic diversity, and the potential for higher yields, making it an effective strategy for addressing local challenges in potato cultivation. Utilizing TPS and other strategies, viz., modern farming practices and novel production technology, can significantly enhance potato production in regions like Pakistan, where spending substantial resources annually on importing seed potatoes is inevitable. This approach is crucial for improving food security, reducing costs, achieving self-sufficiency, and advancing local breeding programs.

Therefore, the promising study sought to evaluate and select potato hybrid cross combinations in their early generations by focusing on heterosis and inbreeding depression. By assessing hybrids for early maturity and yield-attributing traits while emphasizing high heterotic effects and managing inbreeding depression, the study aimed to develop high-performing hybrids well-suited to the high-altitude conditions of Northern Pakistan and similar agroecological regions globally. Additionally, conducting a correlation study to observe associations among different yield-attributing traits is a crucial aspect of potato breeding. This analysis will help identify the key traits influencing tuber yield and other desirable characteristics, guiding more informed selection and breeding decisions.

MATERIALS AND METHODS

Study site

The study transpired in 2022–2024 at the Hazara Agricultural Research Station, Abbottabad (34.1688°N, 73.2215°E), and the Seed Potato Research and Multiplication Farm, Battakundi (34.9316°N, 73.7743°E), Pakistan. The elevations of the experimental sites are 1,256 and 2,812 m above sea level, respectively. Both locations receive substantial rainfall, with annual totals of 1,532 mm in Abbottabad and 1,500 mm in Battakundi. The mean temperatures observed in these areas were 15.9 °C and 3.9 °C, respectively. Soil analysis indicates the soils are slightly acidic, with pH levels of 5.5 in Abbottabad and 6.3 in Battakundi.

Experimental material

Initial crosses among various diverse parental cultivars took place at the Seed Potato Research and Multiplication Farm in Battakundi during the summer irrigation season of 2022 to generate a wide range of genetic variations. Potato berries, containing true potato seed, came from successful cross combinations. The planting of extracted seeds from these berries proceeded in nurseries to grow new potato plants under a greenhouse environment. From there, selecting the most promising plants depended on viability and tuber production, along with desired agronomic traits for further evaluation and testing in different environments. The selected experimental material for this study consisted of seven potato hybrid cross combinations, five parental cultivars, and one check hybrid. The seven different cross combinations included Kuroda × Burna (K × B), Cardinal × Burna (C × B), Kuroda × Roko (K × R), Sarpomira × Roko (S × R), Cardinal × Roko (C × R), Roko × Sarpomira (R × S), and Kuroda × Sarpomira

(K × S). The parental cultivars used for hybridization were Sarpomira, Roko, Cardinal, Kuroda, and Burna. The cross Sarpomira × Kuroda served as a check hybrid in this study. The pedigree of the parents involved in the hybridization is available in Table 1, including information about their genetic background, origin, and breeding history. This information is essential for understanding the genetic composition and potential of the progenies resulting from these crosses.

Experimental design and layout

The experimental design employed was a randomized complete block (RCB) design with three replications, ensuring the robustness and reliability of the results. Planting density was optimal, with a row-to-row distance of 0.75 m and a tuber-to-tuber distance of 0.25 m. Each plot consisted of two rows with 24 tubers, covering a plot size of 4.5 m^2. Both randomization and replication play a significant role in controlling environmental error, reducing bias from unknown variations, and enhancing the reliability and precision of the experiment.

Management of experimental field

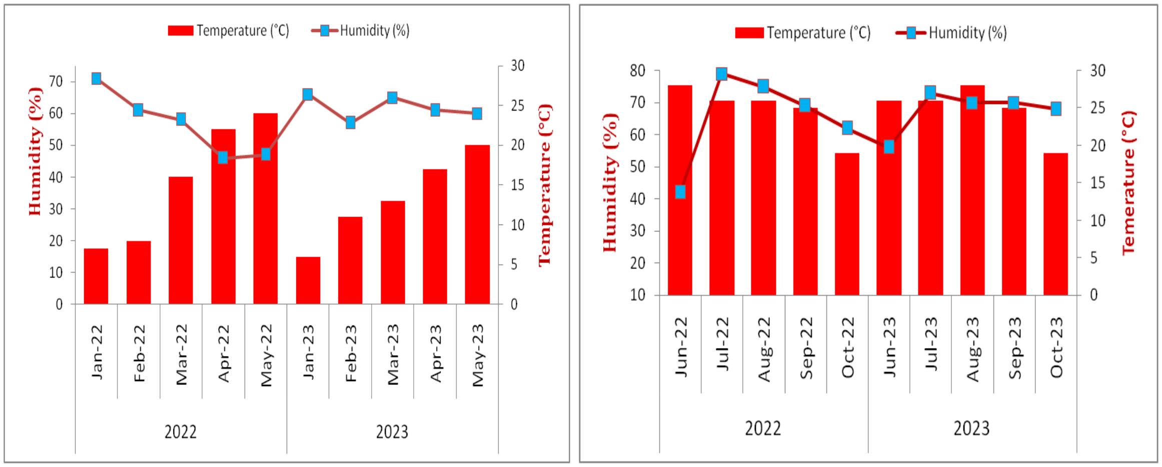
Experimental plots' preparation used proper plowing and a predetermined design. Healthy and well-sprouted seed tubers underwent manual planting. Fertilizers applied were DAP, SOP, and urea at rates of 220, 260, and 180 kg ha^{-1}, respectively. Standard agronomic practices, including timely irrigation and fungicide application, succeeded. Harvesting also proceeded manually to ensure consistent and accurate data collection. All other necessary pre- and post-cultural practices, such as spraying, hoeing, and weeding, occurred in a uniform and timely manner to maintain crop health and vigor throughout the experiments.

Data recorded

Data collected for early maturity and yield-attributing traits included tuber germination percentage, days to maturity, the number of small and medium tubers per plot, and the weight of small and medium tubers per plot (kg) (Cabello *et al.,* 2020). Upon harvesting, the categorization of potato tubers relied on tuber size, i.e., small (25–38 g) and medium (39–75 g) (Asnake *et al.,* 2023). The harvested tubers incurred separating, counting, and weighing in each plot across two environments. The weight of seed-sized tubers used in each experiment was uniform, with a maximum weight difference of about ±2.5 g. These traits provide valuable insights into tuber yield and size distribution, which are critical for potato breeding and production. Additionally, collecting relative humidity (%) and temperature (°C) data occurred, as shown in Figure 1. Monitoring these environmental factors helps assess their impact on the growth and development of potato plants and their tubers. Understanding how environmental conditions influence agronomic traits can inform management practices and breeding strategies, optimizing yield and yield-attributing traits across different growing conditions.

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
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



(A) Abbottabad (B) Battakundi

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

Heterosis

The superiority of offspring in the F_1 generation over their mid-parent, better parent, and standard parent performance refers to the mid-parent heterosis (relative heterosis), better parent heterosis (heterobeltiosis), and standard parent heterosis (standard heterosis), respectively.

Heterosis measurement used the following formulas (Prajapati *et al*., 2023):

$$\text{Relative heterosis (\%)} = F1 - \frac{RH}{RH} \times 100 \quad \text{..........................i}$$

$$\text{Heterobeltiosis (\%)} = F1 - \frac{HB}{HB} \times 100 \quad \text{..........................ii}$$

$$\text{Standared heterosis (\%)} = F1 - \frac{SH}{SH} \times 100 \quad \text{..........................iii}$$

Where,

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

P1 = Mean performance of first parent, i.e., female,
P2 = Mean performance of second parent, i.e., male,
F1 = Mean value of F_1 hybrid, and
BP and SC = Mean performance of better parent and standard check.
Heterosis percentage was categorized into low (0 to 10%), moderate (10 to 20%), and high (greater than 20%).

Inbreeding depression

The inbreeding depression is the reduction or loss in yield, vigor, fertility, and overall production due to inbreeding. The inbreeding depression measurement in the F_2 generation is the percentage decrease of F_2 hybrid performance compared with the mean performance of the F_1 hybrids. The following formula served to measure inbreeding depression (Prajapati *et al*., 2023).

$$\text{Inbreeding Depression (\%)} = F1 - \frac{F2}{F1} \times 100 \quad \text{....iv}$$

Where,
F1 is the performance of inbred generation and F2 is the performance of the hybrid (or out crossed) generation.

T-test

A t-test performed helped determine whether the mid-parent, better parent, and standard parent heterosis were statistically significant. This statistical analysis helps to assess whether the observed differences among the hybrids and their respective parental genotypes for heterosis provide insights into the effectiveness of the hybridization.

The following formula run the t-test (Prajapati *et al*., 2023).

$$t = F1 - \frac{MP}{SE}(F1 - MP) \quad \text{(Relative heterosis)} \ldots\ldots\ldots\ldots\text{v}$$

$$t = F1 - \frac{BP}{SE}(F1 - BP) \quad \text{(Heterobeltiosis)} \ldots\ldots\ldots\ldots\text{vi}$$

$$t = F1 - \frac{SH}{SE}(F1 - SH) \quad \text{(Standard heterosis)} \ldots\ldots\ldots\ldots\text{vii}$$

Where,

MP is the mid parent values, BP is the better parent, and SC is the standard check,

F_1 is the performance of the hybrid generation, and

SE is the standard error.

Statistical analysis

The recorded data underwent the analysis of variance (ANOVA) to assess the significance of differences among treatment means for each environment using Statistix 8.1 software (Analytical Software, Statistix; Tallahassee, FL, USA, 1985-2003). Based on the mean data generated from Statistix 8.1 and the heterosis performance of the traits, genetic variability reached computation for the studied traits of potato hybrid cross combinations. Following ANOVA, applying the least significant difference (LSD) Fisher test (P < 0.05) compared and differentiated between treatment means. The LSD test is a post-hoc analysis used to identify which treatment means are significantly different from one another in the studied environments. A construction of a correlation plot also studied the relationships between different characters. Correlation analysis helps in identifying the strength and direction of these relationships among the studied variables. A positive correlation indicates that two variables tend to move in the same direction, suggesting a positive effect on each other and vice versa. Employing these statistical methods helped the study in comprehensively analyzing the data, identifying significant differences among treatment means, and exploring relationships between various traits. This rigorous analysis provides valuable insights into the performance and interactions of potato hybrids across diverse environments, facilitating the selection and advancement of superior potato hybrid cross combinations in breeding programs.

RESULTS

Analysis of variance

Analysis of variance (ANOVA) revealed significant (P < 0.01) differences among the F_2 population for all traits. Similarly, in the F_1 generation, significant (P < 0.05) differences also appeared among potato hybrids for all traits, except for the tuber germination percentage. This indicates potato hybrids have shown considerable variability, which is essential for further selection and breeding efforts (Table 2).

The potato hybrids developed through the true potato seed (TPS) have displayed promising results for both the overall performance and the potential for future improvement. The significant variability in yield-attributing traits like tuber size and days to early maturity suggests a broad genetic diversity exists within the hybrids, which is a good sign for breeding programs. This variability implies a strong potential for further selection and improvement in future breeding programs.

Heterosis (%)

In potato breeding, the desirability of heterosis varies depending on the specific yield-attributing traits being targeted. The observed ranges for positive relative heterosis (5.73% to 61.78%), heterobeltiosis (2.39% to 61.78%), and standard heterosis (1.38% to 86.01%) are visible in Table 3.

Positive estimates of relative heterosis (RH), heterobeltiosis (HB), and standard heterosis (SH) for tuber germination percentage are favorable in potato breeding.

Table 2. Mean square and coefficient of variations of different traits in studied environments.

Sites	Source	DF	TG (%)	DM	NST/P	NMT/P	WST/P (kg)	WMT/P (kg)
Abbottabad	Replications	2	69.42	3.56	1974.21	199.56	1.87	3.64
	Treatment	13	23.53ns	373.27**	390.64*	285.72**	0.87*	2.74**
	Error	26	16.20	1.81	206.29	32.57	0.44	0.59
	CV (%)	-----	4.29	1.43	12.09	15.26	13.97	17.78
Battakundi	Replications	2	26.50	12.04	744.54	289.09	0.53	0.62
	Treatment	13	33.48**	174.67**	1441.62**	870.29**	1.83**	5.15**
	Error	26	9.36	4.47	163.62	49.50	0.41	0.48
	CV (%)	-----	3.25	2.16	8.57	11.92	10.32	11.17

*, **: Indicates significant at 5% and 1% level of probability, respectively, ns $P > 0.05$ non-significant, CV = Coefficient of variation; TG = Tuber germination%; DM = Days to maturity; NST/P = Number of small tubers plot^{-1}; NMT/P = Number of medium tubers plot^{-1}; WST/P = Weight of small tubers plot^{1}; WMT/P = Weight of medium tubers plot^{-1}.

Table 3. Range observed for relative heterosis, heterobeltiosis, and standard heterosis of various traits in F_1 potato hybrids.

Parameters	Relative Heterosis % (RH)		Number of Significant RH	Heterobeltiosis % (HB)		Number of Significant HB	Standard Heterosis % (SH)		Number of Significant SH
	Min	Max		Min	Max		Min	Max	
TG (%)	-0.39	8.57	0	-4.07	8.32	0	-4.51	1.38	2
DM	-4.65	15.45	0	-4.11	38.17	3	-6.11	28.09	2
NST/P	-21.21	5.73	0	-28.01	2.39	0	-14.91	9.24	6
NMT/P	-24.43	61.78	0	-27.79	61.78	0	-8.86	86.01	7
WST/P (kg)	-39.45	9.21	4	-39.45	6.94	5	-36.87	12.05	2
WMT/P (kg)	-9.21	35.71	8	-18.11	23.91	8	17.71	78.12	5

TG = Tuber germination%; DM = Days to maturity; NST/P = Number of small tubers plot^{-1}; NMT/P = Number of medium tubers plot^{-1}; WST/P = Weight of small tubers plot^{1}; WMT/P = Weight of medium tubers plot^{-1}; Min = Minimum; Max =Maximum.

Estimates of RH, HB, and SH observed were 8.57% (Kuroda × Burna), 8.32% (Kuroda × Burna), and 1.38% (Roko × Sarpomira), respectively (Tables 4 and 5). Meanwhile, negative heterosis is desirable for particular traits in potato breeding, specifically for days to maturity and the number of small tubers per plot^{-1}. The negative heterotic effects recorded for relative heterosis, heterobeltiosis, and standard heterosis for days to maturity and the number of small tubers per plot^{-1} were -4.65% and -21.21% (Cardinal × Burna), -4.11% and -28.01% (Cardinal × Burna), and -6.11% and -14.91% (Kuroda × Roko, and Cardinal × Burna), respectively. More than 50% of the crosses exhibited negative heterotic effects for both the number of small tubers plot^{-1} and days to maturity (Tables 4 and 5).

In potato breeding, achieving positive heterosis for the number of medium tubers plot^{-1} is essential for several reasons, i.e., higher tuber yield, availability of high-quality seed potatoes, and improved marketability. All hybrid cross combinations have demonstrated positive estimates for both relative heterosis and standard heterosis, except for the cross, Cardinal × Burna. The cross combination Cardinal × Roko showed premiere RH, HB, and SH for the number of medium tubers plot^{-1}, with values of 61.78%, 61.78%, and 86.01%, respectively. Conversely, the potato hybrid Kuroda × Roko revealed the lowest RH and SH, with values of 3.65% and 9.86%, respectively (Tables 4 and 5).

For the weight of small and medium tubers plot^{-1}, positive estimates of RH, HB, and SH are crucial for achieving an overall yield per plant, improved quality and quantity of seed potatoes (ensuring better planting material), increased nutritional values, and sustainability

Table 4. Relative heterosis (RH) and heterobeltiosis (HB) of various traits in F_1 potato hybrids.

Traits	Tuber germination (%)		Days to maturity		Small tubers plot^{-1}		Medium tubers plot^{-1}		Small tubers weight plot^{-1} (kg)		Medium tubers weight plot^{-1} (kg)	
F1 hybrids	RH%	HB%	RH%	HB%	RH%	HB%	RH%	HB%	RH%	HB%	RH%	HB%
K × B	8.57	8.32	-3.89	-3.01	-1.57	-9.71	44.29	27.59	1.36	1.36	15.38	0.03
C × B	7.42	7.18	-4.65	-4.11	-21.21	-28.01	-24.43	-27.79	9.21	6.94	-5.55	-13.76
K × R	5.22	3.70	-2.33	1.27	-10.50	-12.96	3.65	-4.45	-18	-19.60	-3.41	-18.11
S × R	-0.39	-4.07	4.83	25.46	-1.75	-7.19	26.24	8.50	7.48	3.26	6.38	6.38
C × R	3.34	1.85	-0.42	4.96	5.73	2.39	61.78	61.78	4.86	-1.30	35.71	23.91
S × K	2.56	-2.70	1.69	16.83	-10.41	-17.50	11.75	-10.22	-39.45	-39.45	5.83	-9.92
R × S	3.87	0.03	15.45	38.17	-11.14	-16.06	8.08	-7.10	0.68	-3.26	-9.21	-9.21
K × S	4.73	-0.65	5.68	21.42	1.30	-6.71	8.84	-12.56	3.40	3.40	-1.66	-16.31

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

Traits	Tuber germination (%)		Days to maturity		Small tubers plot^{-1}		Medium tubers plot^{-1}		Small tubers weight plot^{-1} (Kg)		Medium tubers weight plot^{-1} (Kg)	
F1 hybrids	SH%	ID%	SH%	ID%	SH%	ID%	SH%	ID%	SH%	ID%	SH%	ID%
K × B	-1.04	1.05	-3.45	-12.59	6.71	-25.52	61.04	-73.64	5.67	-35.57	40.62	-66.66
C × B	-2.08	-2.48	-2.69	-12.5	-14.91	-54.78	-8.86	-187.67	9.21	-29.22	23.95	-79.83
K × R	-2.77	1.42	-6.11	-16.59	-9.29	-35.60	9.86	-187.5	-12.76	-62.60	17.70	-88.49
S × R	-2.77	-4.28	16.30	-8.16	8.67	-30.49	73.53	-75.53	12.05	-37.97	56.25	-42.66
C × R	-4.51	-0.36	-2.69	-13.28	6.71	-35.26	86.01	-59.06	7.09	-33.77	78.12	-31.57
S × K	-1.38	2.11	16.30	4.57	-3.39	2.03	43.57	-38.26	-36.87	-80.89	32.29	-27.55
R × S	1.38	-2.73	28.08	0.29	-1.71	-24.57	48.56	-15.12	4.96	-29.05	33.33	-50
K × S	0.69	-0.68	20.86	3.14	9.23	-5.65	39.82	-22.32	7.80	-17.10	22.91	-55.93

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.

in the market. In the presented study, varying degrees of heterosis observed for different cross combinations were 75% and 50% (relative heterosis), 50% and 37.5% (heterobeltiosis), and 75% and 100% (standard heterosis) for both weights of small and medium tubers plot^{-1}, respectively. The hybrid cross combination, Cardinal × Burna, exhibited the ultimate positive RH and HB for the weight of small tubers plot^{-1}, with values of 9.21% and 6.94%, respectively. The cross Sarpomira × Roko showed the maximum SH (12.05%). Moreover, the highest positive estimates of RH, HB, and SH resulted in the cross, Cardinal × Roko, with values of 35.71%, 23.91%, and 78.12%, respectively, for the weight of medium tubers plot^{-1} (Tables 4 and 5).

Inbreeding depression

All hybrid cross combinations have shown a negative inbreeding depression (ID) for the traits, including the number of medium tubers plot^{-1} (-15.12% to -187.67%), weight of small tubers plot^{-1} (-17.10% to -80.89%), and weight of medium tubers plot^{-1} (-27.55% to 88.49%). The highest negative inbreeding depression (-187.67%) for the number of medium tubers plot^{-1} and the lowest ID (-0.36%) for tuber germination (%) emerged in crosses, Cardinal × Burna and Cardinal × Roko, respectively. A significant portion (62.5%, 62.5%, and 87.5%) of cross combinations experienced a negative inbreeding depression for tuber germination (%), days to maturity, and the number of

Table 6. Pearson correlation among yield attributing traits in potato hybrids.

Traits	TG (%)	DM	NST/P	NMT/P	WST/P
DM	0.716**				
NST/P	0.131^{ns}	0.197^{ns}			
NMT/P	0.335^{ns}	0.213^{ns}	0.534*		
WST/P	0.335^{ns}	0.123^{ns}	0.814**	0.558*	
WMT/P	0.454^{ns}	0.248^{ns}	0.545*	0.947**	0.632*

** = $P < 0.01$; * = $P < 0.05$; and ns = $P > 0.05$; TG = Tuber germination, DM = Days to maturity, NST = Number of small tubers plot^{-1}, NMT/P = Number of medium tubers plot^{-1}, WST/P = Weight of small tubers plot^{-1}, WMT = Weight of medium tubers plot^{-1}.

small tubers plot^{-1}, respectively. It was notable that the check hybrid (Sarpomira × Kuroda) has demonstrated a positive ID for tuber germination (%) (2.11%), days to maturity (4.57%), and the number of small tubers plot^{-1} (2.03%), respectively (Table 5).

Correlation

All the studied traits exhibited positive correlations with each other. The number of small tubers plot^{-1} showed a significant positive correlation ($P < 0.01$) with both the weight of small tubers plot^{-1} and the weight of medium tubers plot^{-1} (Table 6). Tubers' germination percentage has demonstrated a positive and highly significant correlation ($P < 0.01$) with days to maturity. Meanwhile, days to maturity highlighted a positive but non-significant correlation ($P > 0.05$) with all the traits, except for tuber germination percentage.

DISCUSSION

Both heterosis and inbreeding depression are important factors in plant breeding, which can be applicable effectively for crop improvement like potato. Achieving high tuber yield, disease resistance, and improving yield-attributing traits are the primary objectives in potato breeding programs. However, achieving these objectives can be quite challenging due to several adverse factors, such as diseases and environmental stresses (drought, temperature), which can significantly affect tuber yield and yield-attributing traits (Mallick *et al*., 2021). The significant ($P < 0.01$, $P < 0.05$) differences among potato genotypes for traits across different environments indicate the presence of heritable variability, broad diversity, and privileged variations, crucial for a successful breeding and selection program. Similar proposed results have come from Asnake *et al*. (2023), Zeleke *et al*. (2021), Dagne *et al*. (2019), and Bekele (2018). Focusing on maximizing the number and weight of small, medium, and large tubers in potato breeding and production can remarkably increase potato yield, improve quality, ensure food security, and foster economic benefits. Therefore, information about genetic variability is vital for making informed breeding decisions, leading to a successful crop improvement and the development of high-performing potato varieties (Ene *et al*., 2016). This variability allows breeders to select individuals with desirable traits and combine them through systematic breeding programs (Bonierbale *et al*., 2020).

Earliness with a high germination rate is a highly desirable trait for both potato breeders and growers, particularly for summer cultivation. Seedling selection is a critical step in potato breeding that directly affects various key traits like yield and disease resistance. Generally, the high discarded rate of progenies in true potato seed (TPS) breeding, often exceeding 90%, can refer to several factors, such as genetic variability, rigorous selection criteria, environmental factors, and substandard performance (Muthoni *et al.,* 2019). By focusing on selecting dynamic and viable seedlings with high heterotic effects, breeders can develop improved potato varieties that can enhance productivity, early maturity, and high tuber number and weight.

Effective seedling selection ultimately contributes to the success and sustainability of potato breeding programs and enhances overall crop performance (Ney *et al*., 2016). Hence, the availability of a population with large genetic diversity is essential for the effective selection of superior varieties with desired traits during breeding programs (Baafi *et al*., 2016).

In potato breeding, the heterosis bears high influences from complex genetic mechanisms, such as multi-allelic gene action (Muthoni *et al.,* 2019). However, selfing can lead to inbreeding depression, impacting yield and fertility (Gopal, 2014). Findings from this promising study indicate the potato hybrids exhibited high mean performance and displayed a wide range of heterotic effects for relative heterosis (-39.45% to 61.78%), heterobeltiosis (-39.45% to 61.78%), and standard heterosis (-36.87% to 86.01%). Heterosis breeding is a valuable technique for improving the characters, viz., yield, maturity, and resistance, which the dominant gene action governs. However, inbreeding depression, along with incompatibility, tetrasomic, and polyploidy nature, had also affected potato hybrids (Ortiz *et al*., 2023). The highest genetic potential for heterosis in terms of tuber yield of cultivated potato showed ploidy and heterozygosity (Muthoni *et al*., 2019). The presence of both lower and higher heterotic effects highlights the complexities of gene expression and inheritance patterns in potato breeding. High heterosis often results from dominant gene action, as reported by Li *et al.* (2022). On the other hand, mini-tubers exhibit little heterosis but can still be more beneficial than micro-tubers produced under controlled conditions artificially (Mallick *et al*., 2021) through tissue culturing. Therefore, the high heterotic performance of potato hybrids, coupled with strong genetic diversity and intense potential, underscores their value in advancing future potato breeding strategies.

Inbreeding depression was noticeably responsible for loss of vigor, fertility, and fitness of the crop if it increased homozygosity and vice versa (Labroo *et al*., 2023). Severe inbreeding depression in cultivated potatoes (diploid/tetraploid), especially when self-pollinated, has linkage to the accumulation of deleterious and dysfunctional alleles (Gopal, 2014). Potato characters, such as tuber yield and yield-attributing traits, have frequently received inbreeding depression influences. The observed positive inbreeding depression in the cross between Sarpomira × Kuroda (check hybrid) for traits, i.e., tuber germination percentage, days to maturity, and the number of small tubers plot^{-1}, highlights the challenges correlated with inbreeding in potato breeding programs. However, the poor agronomic performance of potato hybrids upon selfing in F_2 generation, leading to severe inbreeding depression, was also an outcome, as reported by Almekinders *et al*. (2009). Diploid potato hybrids, with their diverse allelic variations, can play an important role in potato breeding programs when targeting the selection for inbreeding interaction (Lindhout *et al*., 2017). The observed correlations among potato traits provide valuable insights for breeding programs. Significant positive correlations between the number and weight of small and medium tubers and the association between germination percentage and days to maturity offer clear directions for selecting and developing new varieties, as also detailed by Luitel *et al.* (2020).

Substantial amounts of genetic variability, broad diversity, and high heterosis with negligible inbreeding depression in the presented study for different cross combinations have confirmed the role of additive gene action. By selecting and utilizing breeding lines with these characteristics, breeders can develop new varieties that excel in early maturity, yield, and yield-attributing traits. Selecting crosses, such as Cardinal × Roko and Kuroda × Burna, as promising hybrids for their superior performance and desired heterotic effects for yield-attributing traits, along with identifying Battakundi as an ideal environment for true potato seed (TPS) breeding, provides a strong foundation for advancing potato breeding programs. This approach will enhance the overall performance and offer enhanced yield, quality, and crop resilience, contributing to improved productivity and sustainability in Northern

Pakistan and the areas with the same agroecological conditions globally.

CONCLUSIONS

The presence of high genetic variability, broad diversity, heterosis, and little inbreeding depression, as presented by this study, is a significant combination for the success of future potato breeding programs. The cross combinations, specifically Cardinal × Roko and Kuroda × Burna, have been notable as the most promising potato hybrids with the potential for release as new commercial hybrids. Battakundi has emerged as an ideal environment for potato cultivation and for the continuation of potato breeding strategies. By utilizing the true potato seed (TPS) breeding approach in Pakistan, both breeders and farmers have the potential to enhance potato yield, resilience, and quality.

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