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Original Research Article

DEVELOPMENT OF SELECTION CRITERIA FOR EVALUATION OF HOT PEPPER (*CAPSICUM ANNUM* L) GENOTYPE RESILIENT TO CLIMATE CHANGE

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Abstract *The hot pepper chilli, scientifically referred to as Capsicum spp., is globally recognized for its extensive use in cooking and industry. However, rising temperatures due to changing climate conditions are impacting crop yields by affecting pollen viability, as well as the setting and size of the fruit. To combat this, fifteen hot pepper genotypes were assessed to determine which one is most resilient to these climatic challenges. The results indicated that the VRIHP006 genotype exhibited the highest fruit setting and pollen viability percentage among the hot pepper genotypes, with an impressive 86% at optimal temperature and 36.4% under elevated temperatures followed by VRIHP001 and VRIHP004. The data of seed No. fruit-1, fruit length (cm), and fruit width (mm) manifested that genotypes that had less impact of temperature on fruit setting and pollen viability also maintained seed No. fruit-1, fruit length (cm) and fruit width. The Heat susceptibility indices also supported the above selection criteria.*

Keywords pepper; heat stress; genotype; pollen viability; *susceptibility*

Introduction

Hot pepper chilli, known scientifically as Capsicum spp., has a significant global presence due to its widespread culinary and industrial applications. It is a key spice crop, celebrated for its pungency and flavour, enhancing dishes across various cuisines. The worldwide importance of chili peppers extends beyond the kitchen; they are also used in pharmaceuticals, cosmetics, and even self-defense sprays. India stands as the largest producer, consumer, and exporter, contributing approximately 38% to global production and exporting to over 60 countries. The economic value of chili peppers is substantial, with the global value of fresh and dried pepper crops estimated at around $30 billion and $3.8 billion, respectively. The cultivation of hot pepper chili in Pakistan is a significant agricultural activity that contributes to the country's economy. As one of the main red chili-producing countries, Pakistan exported 2,817 metric tons of whole and powdered red chili worth $5,727,738 in 2022-2023. The crop is a high-value cash crop, offering an average net income of $2,344 per hectare, making it a lucrative endeavor for smallholder farmers. The province of Sindh is the major producer, followed by Punjab and Balochistan, with key production clusters in the districts of Badin, Mirpurkhas, and Umerkot (Berry and Rafique-Uddin, 1988).

However, production costs are high due to the labor-intensive nature of harvest and post-harvest processes, which include handling and packaging. Moreover, fluctuations in international market demand and prices can affect the economic stability of chili farming communities. Despite these challenges, the chili pepper industry continues to thrive, adapting through agricultural innovations and market strategies to meet global demand. The crop's versatility and the heat it brings to food and industries alike ensure its enduring significance in the world economy. However, the sector faces several challenges that hinder its full economic potential. Chili cultivation faces challenges such as the need for specific climatic conditions—warm, humid, yet dry weather—and the susceptibility to diseases and pests, which can significantly impact yield and quality. But for most of existed challenges climate change is one of the most effective. The extreme climate conditions such as heatwaves and floods have caused significant crop failures, farmers are struggling to cope with the changing weather patterns (Jifon et al., 2004).

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Cultivating hot peppers necessitates a relatively extended growing period ranging from 130 to 150 days, with fruit production significantly impacted by arid conditions coupled with elevated temperatures. Such high-temperature stress poses a substantial challenge to pepper cultivation. Research indicates that pepper plants thrive and yield optimally when daytime temperatures are maintained between 20 and 30 degrees Celsius. Beyond this range, particularly above 30 degrees Celsius, there is a notable decline in yield, accompanied by the production of substandard fruits. This adverse effect on growth and reproduction is attributed to several physiological and biochemical disruptions within the plant, including accelerated flowering and maturation, which ultimately curtail the plant's life cycle (Tahir *et al*., 2006). The reduction in crop yield due to high temperatures has been linked to damage within the thylakoid membranes, a reduction in chlorophyll levels, and increased cellular membrane leakage. Consequently, cell membrane thermostability has emerged as a valuable physiological marker for identifying heat and drought-resistant varieties within *Capsicum anum* L. species. In the quest for abiotic stress resilience, early developmental stages are often targeted to establish selection criteria, which are hoped to correlate with tolerance during the reproductive phase. Therefore, the current research aims to screen hot pepper germplasm to identify climate-resilient genotypes, suitable for use in hybrid breeding programs.

Materials and Methods

In the 2022-2023 growing season, a comprehensive assessment of heat tolerance was performed on fifteen distinct hot pepper varieties. This study took place at the Vegetable Research Institute in Faisalabad. To protect the chili plants from frost, which they are vulnerable to, a nursery for the genotypes was set up on November 20, 2022, within a controlled tunnel environment. This precaution allowed the seedlings to grow without the danger of frost damage. Once the frost risk subsided, the seedlings, now 8-10 cm tall, were transplanted into the field on February 20, 2023. The field layout was a randomized complete block design, with each genotype replicated three times. The plants were spaced 75 cm between rows and 45 cm between individual plants, arranged on mulch-covered beds. Drip irrigation was the chosen method for watering the plants. Each plot measured 4.5 meters by 0.75 meters. A month after transplanting, the plants underwent earthing up. The fertilization strategy included 66:46:25 kilograms per acre of NPK. Initially, two bags of DAP and one bag of potash were applied during field preparation and transplanting. Additionally, two bags of urea were used throughout the growth stages, with half a bag during earthing up and the rest in 2-3 increments at different growth phases.

In this study, data collection was conducted on a set of ten plants chosen at random to represent each variety under examination. The parameters measured included the percentage of fruit set, the rate of seed formation, as well as the length and diameter of the fruits, measured in centimeters and millimeters respectively. These observations were systematically recorded on different dates of the year 2023. It was noted that the highest and lowest percentages of fruit set across all genotypes tested coincided with the first week of April and the second week of June, respectively. This variation aligns with the prevailing temperature conditions, with the optimal temperature recorded as 34/16 °C (day/night) in April and a higher temperature of 40/24 °C (day/night) observed in June. The fruit set percentage of each genotype was assessed by tagging twenty flowers and it was computed by using the following formula:

Number of set fruits Fruit set %= total number of flowers tagged×100

The assessment of pollen viability was conducted using a 2% acetocarmine staining technique. Under microscopic examination, viable pollen grains took on a range of hues from pink to a striking red, while those that were non-viable remained unstained and thus appeared transparent. This staining method provides a clear visual distinction that aids in determining the fertility potential of the pollen samples. The percentage of pollen viability was calculated by using the following formula:

Number of viable pollen grains

Pollen viability %=total number of pollen grains×100

In a meticulous examination, fruits were dissected to determine the count of seeds per fruit. Measurements of length and diameter were meticulously taken from a random selection of mature green fruits, utilizing a centimeter scale for length and a vernier caliper for diameter, respectively. This methodical approach ensures precise data collection for further analysis in horticultural studies. The calculation of heat susceptibility indices, as outlined by Fischer and Maurer in 1978, encompasses a range of factors including fruit set, pollen viability, the number of seeds per fruit, as well as the fruit's length and diameter. To determine the heat susceptibility index specifically for the percentage of fruit set, a distinct formula is employed. This methodical approach allows for a comprehensive assessment of how heat impacts various aspects of fruit development, providing valuable insights for agricultural practices in environments subject to high temperatures. The heat susceptibility index for the fruit set percentage was calculated by using the following formula:

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HSI=(1-Y/Yp) D-1

In this study, Y=represents the percentage of fruit set in a genotype when subjected to high temperatures (40/24 °C, day/night), while 'Yp' denotes the percentage of fruit set in the same genotype under optimal temperature conditions (34/16 °C, day/night). The stress intensity 'D' is calculated using the formula (1-X/Xp), where 'X' is the average 'Y' across all genotypes, and 'Xp' is the average 'Yp' for all genotypes. The Heat Susceptibility Index (HSI) was similarly determined for various parameters. To ascertain the critical difference values, an analysis of variance (ANOVA) was conducted, following the methodology outlined by Singh et al. (1991).

Results and Discussion

Under the optimal temperature conditions of 34/16 °C (day/night), the VRIHP006 genotype exhibited the highest fruit setting percentage among the hot pepper genotypes, with an impressive 86% at optimal temperature and 36.4% under elevated temperatures. This was closely followed by the VRIHP001 genotype, which showed an 83.1% fruit set at optimal temperature and 25.2% under high temperatures. Conversely, the VRIHP0013 genotype demonstrated the lowest fruit set percentages, with only 18.3% under optimal conditions and a mere 2.8% at higher temperatures, as detailed in Table 1. The production of sufficient viable pollen is crucial, as its deficiency has been linked to reduced fruit set and a lower number of seeds per fruit in Capsicum species, particularly when high temperatures overlap with the flowering period, as noted by Schoper *et al*., (1987) and Reddy and Kakani, (2007). For instance, the pollen viability for VRIHP001 plummeted from 80% at the optimal temperature to just 28% under high temperatures. The range of pollen viability for hot pepper genotypes at optimal temperature spanned from 80 to 100%, according to Table 1. However, at higher temperatures, all hot pepper genotypes showed a variation in pollen viability of 60% or greater. This suggests that the decline in fruit set at elevated temperatures in hot peppers may be attributed to disruptions in processes other than pollen viability, such as pollen germination or fertilization.

Elevated temperatures were found to not only impede fruit set but also impair fruit quality, evidenced by a decrease in the number of seeds per fruit, as well as reductions in fruit length and diameter. For instance, under optimal temperature (OT) conditions, the seed count per fruit varied from 69.7 in the VRIHP006 variety to 35.2 in VRIHP013, with an average of 49.4. In contrast, under high temperature (HT) conditions, this range was 55.4 to 9.4, respectively, with the average plummeting to 25.5. The most significant decline in seed count per fruit was seen in VRIHP013, with a drastic 81.8% drop, while VRIHP012 experienced a 62% reduction. Conversely, VRIHP006 exhibited the least reduction at 21.1%, followed closely by VRIHP001 at 27.4%. Specifically, the Royal Wonder variety showed a seed count per fruit of 8.0 at OT, which fell to 2.0 at HT. It's noteworthy that a high seed count during periods of heat stress in tomatoes is a crucial measure of heat tolerance, as heat stress typically leads to a marked decrease in the production of viable seeds (Berry and Rafique-Uddin, 1988). In the observed data, genotype VRIHP003 stood out with the largest fruit dimensions under optimal temperature conditions, as detailed in Table 2. Conversely, genotype VRIHP006 demonstrated remarkable resilience to high temperatures, showing the least shrinkage in fruit size, with a modest 17.7% decrease in length and a 7.1% decrease in width. On the other end of the spectrum, genotype VRIHP013 was the most affected by heat, with a drastic reduction in fruit dimensions, shrinking by 91.1% in length and 54.3% in width. The Heat Susceptibility Index (HSI) served as a critical metric for assessing the heat tolerance of the hot pepper germplasm. This index facilitated the identification of genotypes that sustained minimal impact on crucial parameters such as fruit set percentage, pollen viability, seed count per fruit, and overall fruit dimensions in response to elevated temperatures (Tahir *et al*., 2006). According to the Heat Susceptibility Index (HSI) related to the fruit set, genotype VRIHP006 emerged as highly heat tolerant. Meanwhile, genotypes VRIHP001 and VRIHP004 were identified as moderately heat tolerant, with their HSI ranging from 0.5 to 1.0. Genotypes with an HSI greater than 1.0 were deemed heat-sensitive. Notably, genotypes VRIHP006, VRIHP001, and VRIHP004 exhibit promising characteristics for initiating a hybrid breeding program aimed at developing heat-tolerant chili cultivars, as evidenced by their superior fruit set under elevated temperatures (HSI<1.0). In terms of pollen viability, genotype VRIHP006 was categorized as highly heat tolerant with an HSI of less than 5, while the other genotypes, VRIHP001 and VRIHP004, were classified as moderately heat tolerant, with an HSI ranging from greater than 0.5 to less than 1.0, as reported by Pradhan et al. in 2012. The increasing global temperatures due to climate change are anticipated to pose significant challenges to the commercial cultivation of vegetable peppers in the future (Gajanayake etal., 2011). The emphasis should be needed to develop cultivars with higher heat tolerance (Jifon et al., 2004).

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Table 1: Influence of temperature on fruit set and pollen viabilityof different hot pepper genotypes

Genotype	Fruit set (%)			Pollen vi-ability (%)		
	OT	HT	HSI	OT	HT	HSI
VRIHP006	86.0	66.4	0.8	90	80	**0.61**
VRIHP001	83.1	55.2	0.97	90	80	**0.61**
VRIHP004	81.9	52.0	1.18	100	80	**1.11**
VRIHP005	80.0	49.4	0.96	80	70	**0.67**
VRIHP008	77.9	48.0	0.94	90	85	**0.33**
HF-86	72.6	46.9	1.03	100	60	**0**
VRIHP003	67.6	46.7	1.29	80	60	**1.39**
VRIHP007	63.3	46.0	0.18	80	50	**0**
VRIHP002	61.9	42.8	1.18	100	50	**1.11**
Golden Hot F1	59.4	41.5	1.39	90	70	**1.22**
VRIHP009	56.0	41.0	1.39	80	70	**0.67**
VRIHP010	53.3	39.8	1.10	100	30	**3.89**
VRIHP011	52.3	18.9	1.12	90	28	**3.92**
VRIHP012	50.1	13.3	1.15	85	28	**3.95**
VRIHP013	**48.3**	**6.8**	**1.15**	**85**	**28**	**3.95**

Table 2: Influence of temperature on fruit quality of different hot pepper genotypes

Genotype	Seed No. fruit^{-1}			Fruit length (cm)			Fruit width (mm)		
	OT	HT	HSI	OT	HT	HSI	OT	HT	HSI
VRIHP006	69.7	55.0	0.50	4.8	3.95	0.27	11.41	10.6	**0.68**
VRIHP001	69.0	50.1	0.64	4.8	3.7	0.33	10.43	9.6	**0.68**
Golden Hot F1	69.8	45.0	0.84	4.1	3	0.39	13.11	10.5	**0.70**
VRIHP005	57.3	40.5	0.99	5.6	3.9	0.4	11.95	10.1	**0.72**
VRIHP008	57.2	36.0	0.99	4.6	2.9	0.48	11.76	9.5	**0.79**
VRIHP004	54.0	31.0	1.14	4.9	2.9	0.51	14.31	9.8	**0.86**
VRIHP002	52.5	30.0	1.19	6.3	3.5	0.67	11.1	7.29	**1.10**
VRIHP007	51.0	26.0	1.24	5.7	3.1	0.7	13.96	9.1	**1.19**
HF-86	47.7	25.5	1.60	5.7	3.1	0.77	10.45	6.3	**1.61**
VRIHP003	45.7	24.7	1.88	6.6	3.1	0.77	16.83	10.1	**0.84**
VRIHP009	42.8	23.6	2.04	6.5	2.9	2.33	13.1	7.6	**2.23**
VRIHP010	40.0	21.0	2.53	4.7	1.5	2.87	13.1	7.5	**2.68**
VRIHP011	38.2	18.4	3.5	4.7	0.8	2.88	11.2	6.1	**2.71**
VRIHP012	37.1	14.1	4.1	4.7	0.5	2.89	10.1	5.4	**2.88**
VRIHP013	**35.2**	**6.4**	**4.5**	**4.5**	**0.4**	**2.9**	**9.2**	**4.2**	**2.98**

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Declaration

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

The study was approved by authors.

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Conflict of Interest

There is no conflict of interest among the authors regarding this case study.

Authors Contribution

All authors contributed equally.

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