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Putrescine enhances heat stress tolerance in *Brassica juncea* L. Seedlings by modulating growth and physiological responses

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Abstract
Heat stress resulting from the increasing global temperatures is emerging as a primary constraint on crop productivity and negatively affects plants. Polyamines (PAs), small, positively charged nitrogenous compounds, play essential roles in plant growth, development, and stress responses. Putrescine (Put) functions as a vital growth regulator, promoting both plant growth and resistance to stress. The primary objective of the present research was to explore the mechanisms through which Put (1 mM) mitigates the adverse effects of heat stress on genotypes RH-1707, RH-1708 (thermosensitive) and RH1566 and RH-1999-42 (thermotolerant). Heat stress led to reduction in growth attributes such as shoot length, root length, fresh weight, dry weight, germination percentage, chlorophyll content and relative water content (RWC) whereas it increased membrane damage as was seen by a rise in electrolyte leakage and lipid peroxidation. The application of Put helped alleviate the adverse effects of heat stress by reducing lipid peroxidation and electrolyte leakage whereas boosting chlorophyll content, RWC and growth parameters. Most significant role of Put in reducing the detrimental effects of heat stress was observed in RH-1707 and RH-1708, which were more susceptible to cellular damage under heat stress. This study specifies that Put foliar spray (1mM) is effective in mitigating the effects of heat stress in *Brassica juncea* L.

Keywords: Heat stress, *Brassica juncea* L., polyamines, putrescine, growth

Introduction

The *Brassicaceae* family encompasses a broad range of plants, offering an extensive and diverse range of end products derived from a single plant genus, which are widely utilized by humans. Mustards, part of the *Brassicaceae* family, are among the earliest cultivated plants and have been utilized for centuries as vegetables, condiments, and for the production of industrial oils (Raboanatahiry *et al.*, 2021) [46]. Among nine major oilseeds Soybean (34%), Mustard (31%) and Groundnut (27%) contributes to more than 92% of total oilseeds production in India. However, in terms of vegetable oil production Mustard, Groundnut, and Soybean contributes 31.49%, 19.81% and 17.99% respectively. In India, rapeseed-mustard occupies 6.69-million-hectare area with production 10.11 million tonnes and yield of 1676 kg/ha (Chauhan *et al.*, 2021) [13]. Major rapeseed producing states are Rajasthan, Madhya Pradesh and Haryana. In Haryana, total production is 4.5 million tonnes with 70.19% cumulative share of production. India holds the 2nd position globally in acreage and 4th in production of rapeseed mustard (DRMR, 2023) [18].

Plants being immobile gets significantly affected by various abiotic environmental factors leading to a variety of responses, including alterations in biological processes such as gene expression and cell metabolism, as well as impacting overall growth and development (Zhang *et al.*, 2023) [67]. These abiotic stress factors comprise of extreme temperature variations, drought, flooding, salinity, metal exposure, and nutrient deficiencies. Each of these stresses causes different types of responses in plants (Radha *et al.*, 2023) [47]. Since the beginning of the century, a noticeable rise in ambient temperatures has been recorded, with projections indicating a continued upward trend as a result of climate change (Pandey *et al.*, 2019) [43]. An elevation in temperature that surpasses the threshold level by even one degree is classified as heat stress

(Hasanuzzaman *et al.*, 2013) [27]. Each degree centigrade increases in average growing season temperature reduce *Brassica* crop yield by 17% (Lobel and Asner, 2003) [36]. Heat stress affects plant growth throughout all developmental stages, with the threshold for heat tolerance varying considerably at different growth phases. This stress negatively influences several physiological processes, including photosynthesis, respiration, water balance, and membrane stability (Ahmad *et al.*, 2022) [1]. Being a rabi crop, Indian mustard still faces high temperature at different stages due to uncertainties in climate. Elevated temperatures have the potential to either decelerate or entirely terminate the germination process (Venkateswarlu and Prasad, 2012) [61]. High temperatures during the sowing period can impede seed germination and result in the death of seedlings, ultimately causing uneven crop distribution and reduced seed yields (Azharudheen *et al.*, 2013) [7].

A key approach in mitigating heat stress in plants is the application of phytohormones, osmoprotectants, and polyamines (PAs) (Shao *et al.*, 2022) [51]. Polyamines constitute a category of small, polycationic, aliphatic metabolites that are present universally in all living organisms. They play crucial roles in various physiological processes (Bohra *et al.*, 2015) [10]. Their cationic nature is key to their biological functions. Due to their positively charged nature at physiological pH, polyamines have the ability to interact with phospholipids in the membranes as the amine groups of polyamines form favourable electrostatic interactions with the negatively charged phosphate groups in the membrane (Falasca *et al.*, 2010) [22]. Putrescine (Put) serves as the key product in the polyamine biosynthesis pathway (Tiburcio *et al.*, 2014) [58]. Importantly, Put plays a crucial role in imparting tolerance to diverse abiotic stresses (Walters, 2003) [63]. Put's capacity to induce responses that enhance tolerance to abiotic stress is linked to several key processes, including the scavenging of free radicals, modulation of ABA levels, prevention of lipid peroxidation, preservation of cellular pH and ionic equilibrium, and regulation of cationic channels (Gill and Tuteja, 2010) [24]. The efficacy of polyamines, in alleviating the detrimental effects of high temperature has been thoroughly examined (Asthir and Deep, 2011; Hassanein *et al.*, 2013; Das *et al.*, 2021; Amooaghaie and Moghym, 2011) [6, 28, 15, 5]. Nevertheless, the precise contribution of polyamines in alleviating heat stress in *B. juncea* has not been extensively studied. Hence, this investigation was undertaken to explore the acclimation response of *B. juncea* seedlings when subjected to heat stress exposure by exogenous application of Put.

Material and methods

Four Indian mustard (*Brassica juncea* L. Czern. & Coss.) genotypes RH-1707, RH-1708, RH-1566 and RH-1999-42 were utilized for the study. Seeds were obtained from Oilseeds Section, Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. Uniformly selected seeds were surface sterilized with 0.1% $HgCl_2$ for one minute and then repeatedly washed with distilled water. 20 seeds from each genotype were grown in triplicates in Petri plates under controlled conditions (light- 75 W/m^2, 70% RH, temperature 25 ± 2 °C) in the growth chamber. After 10 days, seedlings were subjected to heat treatment by gradually elevating the temperature to 40-42 °C (RH ~ 50-65%) for 3 hr in light. The temperature was reduced to 25 °C (RH ~ 70%) after 3 hr, and the cycle was repeated for 4 days. In contrast, a control experiment was carried out where the optimal temperature (25 °C, RH ~ 70%) was maintained throughout the experiment. Put (1mM) was given as pretreatment to the seedlings 2 hr before exposing them to heat stress. Similarly, Put (1mM) was given to seedlings kept at the optimal temperature. There were four distinct treatments used in the experiment- (1) Control, (2) Put (1 mM), (3) Heat (40–42 °C) and (4) Heat + Put (1 mM).

Germination percentage

Healthy uniform seeds were sterilized using a 0.1% $HgCl_2$ solution and rinsed multiple times with distilled water. 20 sterilized seeds were then placed in Petri plates for germination at a temperature of 25 ± 2 °C for a period of 3 days. Heat treatment involved exposing the seeds to temperatures between 40-42 °C for 3 hr, while Put treatment involved soaking the seeds in a Put (1mM) solution for 8 hr before transferring them to Petri dishes. The percentage of seeds showing standard radicle emergence after 3 days was recorded as the germination rate.

Determination of Growth Attributes

Various growth parameters, including shoot length, root length, fresh weight, and dry weight, were assessed. Seedlings length was measured using a standardized scale (in cm). The fresh weight of seedlings was recorded, and thereafter, they were subjected to drying in an oven at 65 °C for 48 hours to determine their dry weight.

Chlorophyll content

50 mg of fresh tissue was taken in triplicates and washed thoroughly. Then they were kept in 5 ml of methanol overnight for extraction of chlorophyll. The absorbance levels were measured at wavelengths of 653 nm, and 666 nm (Nobosse *et al.*, 2018). Total chlorophyll was calculated using the equations:

Chlorophyll *a* = (15.65OD666 – 7.34OD653) × V/1000

Chlorophyll *b* = (27.05OD653 – 11.21OD666) × V/1000

Chlorophyll content = Chlorophyll *a* + Chlorophyll *b*

Relative water content (RWC)

Leaf RWC was determined following the method outlined by Smart *et al.* (1974). 100 mg of fresh sample was collected in triplicate. These leaves were then placed individually in petri dishes with distilled water overnight to achieve full turgidity. Afterward, the turgid leaves were re-weighed, and subsequently dried in an oven at 70 °C for 72 hours. The dried samples were then used to calculate the RWC by the formula:

RWC (%) = (Fresh weight − Dry weight/Turgid weight − Dry weight) × 100

Electrolyte leakage and membrane stability index

The relative intactness of plasma membrane was measured using a conductivity meter as the leakage percentage of electrolytes, as described by Dionisio-Sese *et al.* (1998) [17]. 50 mg fresh tissue was cut into smaller pieces and placed in test tubes immersed in 20 ml of distilled water, where it was left overnight. The initial electrical conductivity of the solution (EC1) was measured the next day. Subsequently, the samples were autoclaved for 20 minutes to release all electrolytes, cooled, and the final electrical conductivity (EC2) was recorded. The percentage of electrolyte leakage was then calculated as follows:

Electrolyte Leakage (%) = (EC1/EC2) × 100

Lipid peroxidation

The degree of lipid peroxidation was assessed by measuring malondialdehyde (MDA) concentration found in leaf tissues. MDA, a byproduct of lipid peroxidation, was detected through

the method given by Heath and Packer (1968). Firstly, 0.5 ml of supernatant was added to 2.3 ml of 20% (w/v) trichloroacetic acid (TCA) solution containing 0.5% thiobarbituric acid (TBA). This mixture underwent heating in a water bath at 95 °C for 30 minutes followed by rapid cooling in an ice bath. The absorbance of the reaction mixture was measured at 532 nm and 600 nm.

Results

Germination percentage: The germination percentage was determined by assessing 20 seeds, and it was noted that the highest decrease in germination percentage caused by heat stress in case of RH-1708 (47.3%) and RH-1707 (40%). Tolerant genotypes exhibited a decline of 15.7% in RH-1566 and 21% in RH-1999-42 under heat stress. Application of Put during heat stress significantly enhanced seed germination across all genotypes studied, with RH-1708 showing the most substantial improvement at 44.4%. Notably, there were no significant changes in the germination % between the control group and those treated with Put (Fig. 1, 2).

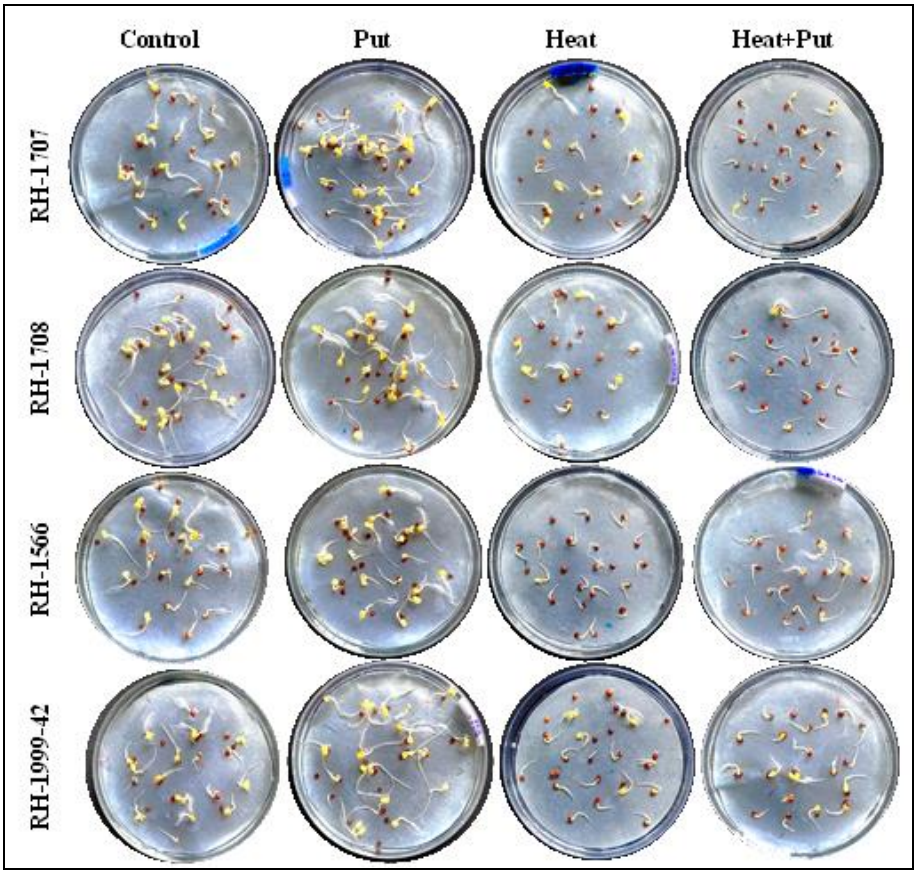

Fig 1: Effects of heat stress and putrescine application on germination percentage of *Brassica juncea* L.

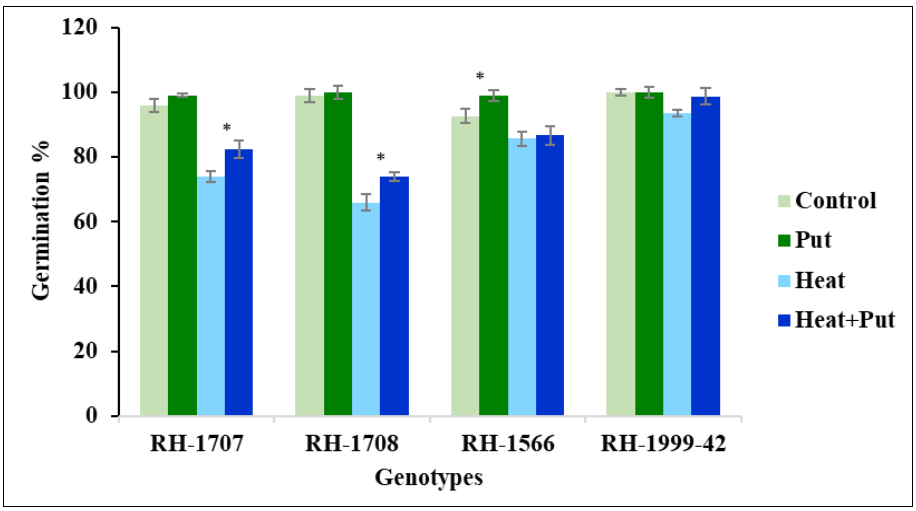

Fig 2: Effects of heat stress and putrescine application on germination percentage of *Brassica juncea* L. grown in Petri plates. Each bar represents the mean (n = 3) and the error bar indicates the standard deviation. Asterisk (*) indicates significant difference between the treatments

Shoot length

A significant decrease in shoot length was noticed in RH-1708 (41%) and RH-1707 (30.7%); however, a lesser decrease was shown by RH-1566 (22.5%) and RH-1999-42 (19.1%). The application of Put under normal temperature showed a very slight increment in shoot length. The application of Put

mitigated the effects of heat stress, leading to improvements in RH-1708 (21.4%), Rh-1707 (18.7%), RH-1999-42 (17.3%) and RH-1566 (11.3%). (Fig. 3, 4, Table 1).

Root length

The impact of heat stress on root length was most pronounced in RH-1708 (42.8%) and RH-1707 (40%), while the reduction was less pronounced in tolerant genotypes RH-1566 (26.8%) and RH-1999-42 (29%). Additionally, the application of Put increased root length significantly under heat stress, with RH-1708 showing the highest increase (17.6%), followed by RH-1566 (13.2%), RH-1707 (7.7%), and RH-1999-42 (6.6%). (Fig. 3, 4, Table 1).

Fresh weight

The fresh weight of seedlings reduced under heat stress as observed in RH-1708 (55.2%) and RH-1707 (41.4%), in contrast to RH-1566 (29.9%) and RH-1999-42 (26.2%). When Put was applied alongside heat stress, it mitigated the effects of heat stress by improving the fresh weight of seedlings, with the most significant improvement observed in RH-1707 (29%), followed by RH-1708 (20.9%), RH-1999-42 (11.3%), and RH-1566 (10%). (Table 1).

Dry weight

Similar to fresh weight, the dry weight of seedlings was also reduced under heat stress in all the studied genotypes, with the most significant reduction observed in RH-1708 (45%) and RH-1707 (35.6%), followed by RH-1566 (23.1%) and RH-1999-42 (19.5%). The application of Put improved the dry weight of seedlings under heat stress, with the most significant improvement observed in RH-1708 (19.7%) and RH-1707 (17.8%), followed by RH-1999-42 (13.5%) and RH-1566 (10.5%). (Table 1).

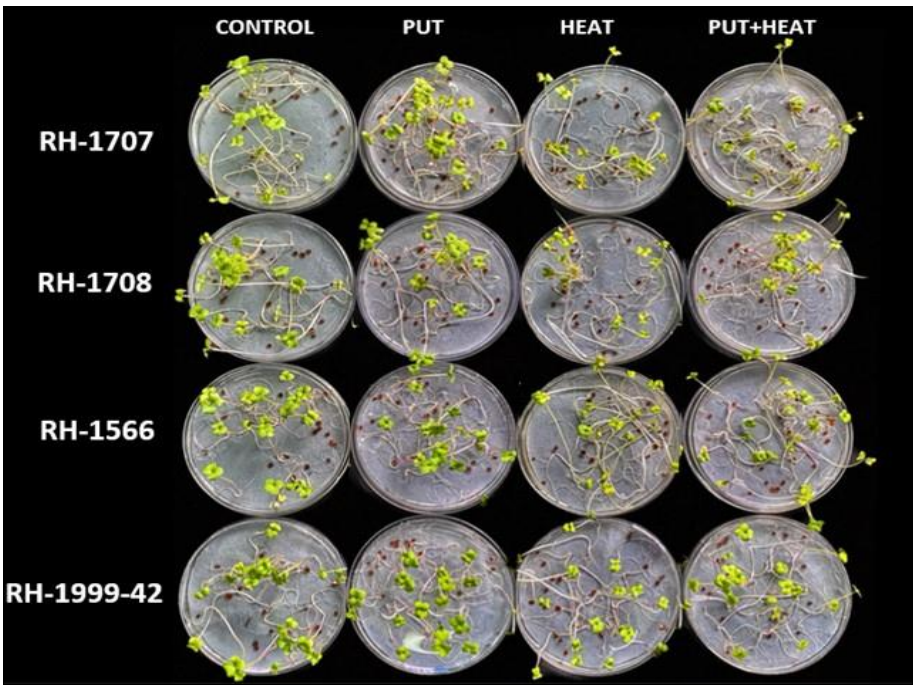

Fig 3: *Brassica juncea* L. seedlings under the effects of heat stress and putrescine application

Table 1: Effects of heat stress and putrescine application on growth parameters in *Brassica juncea* L. seedlings grown in Petri plates

		Shoot length (cm)	Root length (cm)	Fresh weight (gm)	Dry weight (gm)
RH-1707	Control	5.40	6.69	1.18	0.05
	Put (1mM)	5.78	6.99	1.29	0.06*
	Heat (40-42°C)	3.74	3.82	0.69	0.03
	Heat+Put	3.97*	4.12*	0.89*	0.04*
RH-1708	Control	6.24	6.25	1.21	0.06
	Put (1mM)	6.30	6.87*	1.43*	0.07
	Heat (40-42°C)	3.62	3.68	0.52	0.03
	Heat+Put	3.81*	4.21*	0.62*	0.04*
RH-1566	Control	5.81	6.07	1.12	0.06
	Put (1mM)	6.05	6.40	1.39*	0.07
	Heat (40-42°C)	4.33	4.44	0.79	0.04
	Heat+Put	4.44	5.02	0.87	0.05
RH-1999-42	Control	5.81	6.45	1.20	0.06
	Put (1mM)	6.15*	7.20*	1.31	0.08*
	Heat (40-42°C)	4.64	4.58	0.88	0.05
	Heat+Put	4.96*	4.88	0.98	0.06
	CD (P=0.05)	*G= 0.09, T=0.311, GxT=0.401*	*G= 0.121, T= 0.341, GxT=0.462*	*G= 0.12, T= 0.281, GxT=0.401*	*G= 0.005, T= 0.01, GxT=0.015*

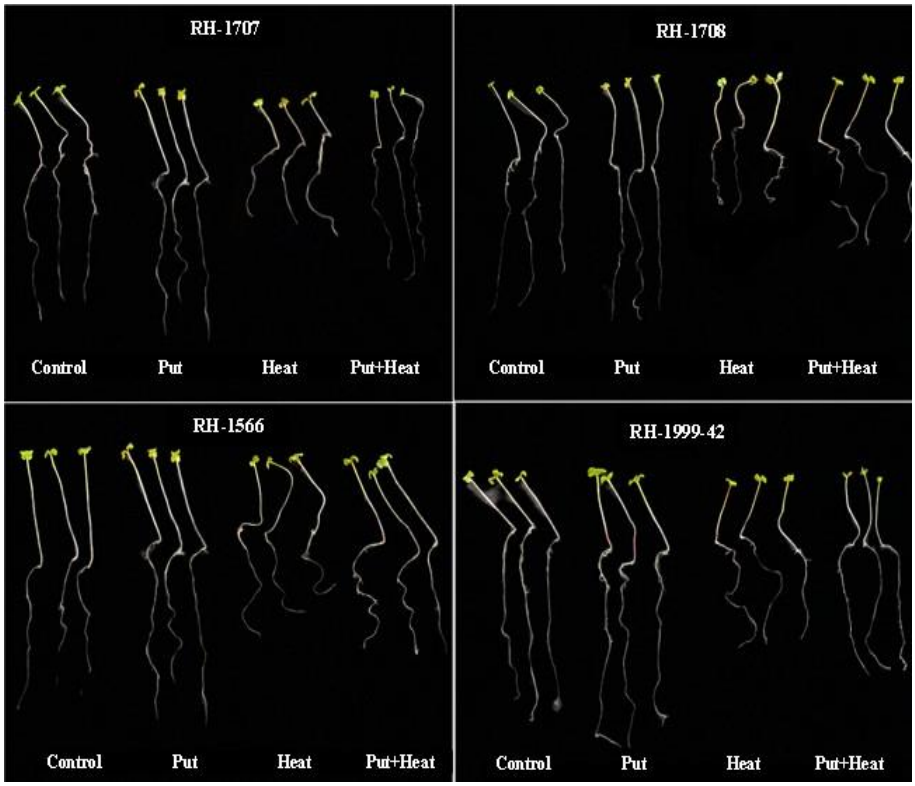

Fig 4: Effects of heat stress and putrescine application on shoot and root length of *Brassica juncea* L.

Chlorophyll content

Heat stress leads to a sharp decline in chlorophyll content in genotypes RH-1708 (41.59%) and RH-1707 (36.12%) whereas RH-1566 (18%) and RH-1999-42 (16.9%) were less affected. Under controlled conditions, exogenous application of Put led to a slight increase in chlorophyll content in RH-1707 (8.9%), RH-1708 (15.4%), RH-1566 (5.9%) and RH-1999-42 (11.56%). However, combined treatment of both Put and heat stress led to a considerable improvement in chlorophyll content as was seen in RH-1707 (21.76%), RH-1708 (25.16%), RH-1566 (15.28%) and RH-1999-42 (11.57%). (Fig. 5).

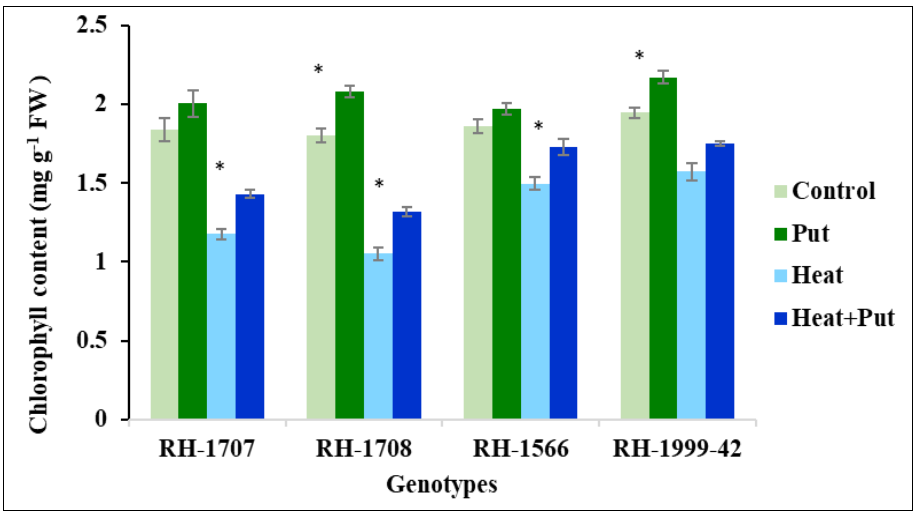

Fig 5: Effects of heat stress and putrescine application on chlorophyll content of *Brassica juncea* L. grown in Petri plates. The error bar indicates the standard deviation and each bar of graph represents the mean (n=3). Asterisk (*) indicates significant difference between the treatments

Relative water content

Under heat stress, the RWC content was significantly reduced, more substantially in genotypes RH-1708 (38.63%) and RH-1707 (31%) whereas genotypes RH-1566 (10.23%) and RH-1999-42 (15.4%) showed a lesser decline. The exogenous application of Put at normal temperature led to a slight increase in RWC in genotypes RH-1707 (10.47%), RH-1708 (3.35%), RH-1566 (9%) and RH-1999-42 (1.91%). However, under heat stress, Put application shows a more efficient increase in the water status of plants, as was seen in genotypes RH-1707 (17.36%), RH-1708 (20.3%), RH-1566 (3.11%) and RH-1999-42 (6.74%) (Fig. 6).

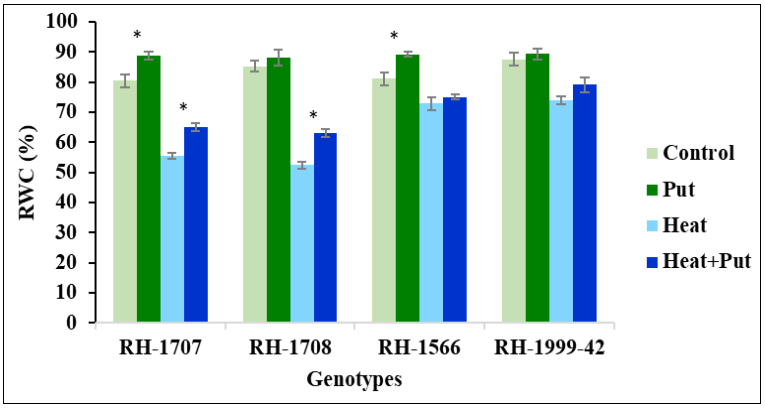

Fig 6: Effects of heat stress and putrescine application on RWC of *Brassica juncea* L. grown in Petri plates. The error bar indicates the standard deviation and each bar of graph represents the mean (n=3). Asterisk (*) indicates significant difference between the treatments

Electrolyte leakage

All the studied genotypes experienced an elevation in electrolyte leakage, but a more significant increase was noticed in genotypes RH-1708 (33.96%) and RH-1707 (28%) than in RH-1999-42 (9.3%) and RH-1566 (17.86%). The exogenous application of Put at normal temperature led to a slight increase in electrolyte leakage in genotypes RH-1999-42 (18.53%), RH-1707 (9%), RH-1566 (7.6%) and RH-1708 (11.43%). The damage caused to membranes due to heat stress was considerably reduced by exogenous application of Put, as was seen in genotypes RH-1707 (14.71%), RH-1708 (22.45%), RH-1566 (2.56%) and RH-1999-42 (7.52%) (Fig. 7).

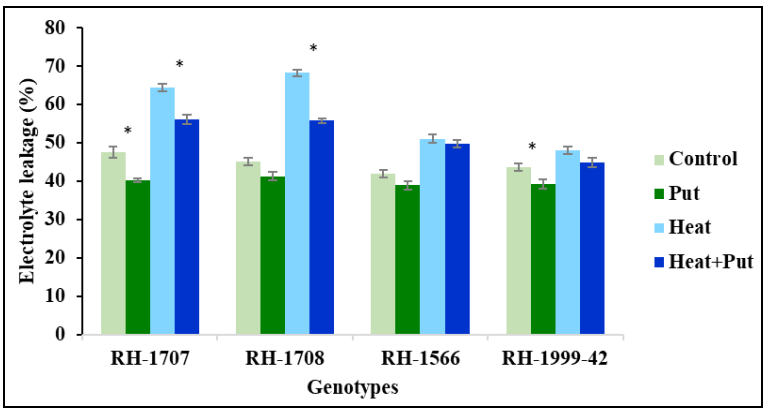

Fig 7: Effects of heat stress and putrescine application on electrolyte leakge of *Brassica juncea* L. grown in Petri plates. The error bar indicates the standard deviation and each bar of graph represents the mean (n=3). Asterisk (*) indicates significant difference between the treatments

Lipid peroxidation

Lipid peroxidation was assessed by measuring MDA content. Under heat stress, all the studied genotypes exhibited an increase in MDA content, with the maximum increase in RH-1708 (47.39%) and RH-1707 (34.42%). RH-1566 (12.67%) and RH-1999-42 (5.11%) showed an increase but to a lesser extent. Under normal temperature, the application of Put resulted in a more efficient reduction of MDA levels in genotype RH-1707 (12.86%) in comparison to RH-1708 (10.85%), RH-1566 (9.77%), and RH-1999-42 (6.8%). The exogenous application of Put in combination with heat stress resulted in a reduction of MDA content in RH-1707 (8.44%), RH-1708 (16%), RH-1566 (12.31%) and RH-1999-42 (5.3%) (Fig. 8).

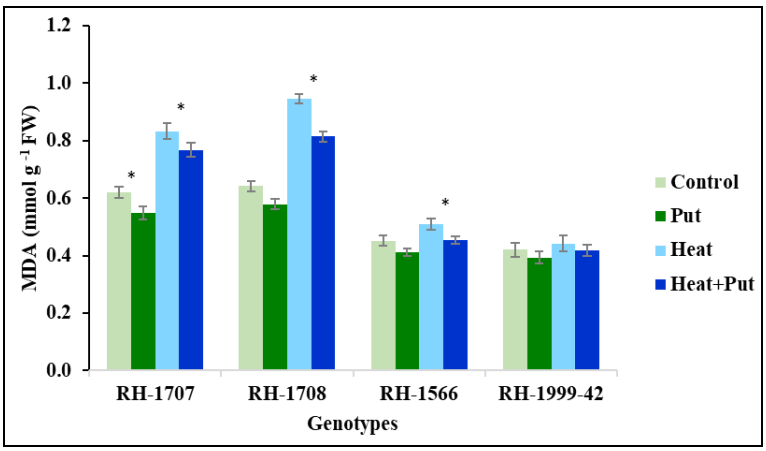

Fig 8: Effects of heat stress and putrescine application on lipid peroxidation of *Brassica juncea* L. grown in Petri plates. The error bar indicates the standard deviation and each bar of graph represents the mean (n=3). Asterisk (*) indicates significant difference between the treatments

Discussion

Steadily rising ambient temperature is one of the most harmful stresses for plant growth and development. Heat stress exerts a harmful impact on plants by disturbing their metabolic processes, subsequently affecting their growth, physiological, biochemical traits, and yield attributes either directly or indirectly (Brar *et al.*, 2022) [11]. Plants employ a variety of strategies to cope with heat stress which includes preserving the integrity of their cell membranes, eliminating harmful ROS, generating antioxidants, accumulating and adapting compatible solutes, and triggering kinase cascades (Pandey and Singh, 2024) [44]. In recent years, the use of growth regulators, also known as bio stimulants, has been conducted as a strategic approach in agronomic management to reduce the adverse impacts induced by environmental stress (Akhtar *et al*., 2022) [33]. Various studies have demonstrated that polyamines exhibit multifaceted roles in plant response and stress tolerance. These include maintaining elevated chlorophyll levels, reducing levels of ROS, preserving the integrity of plasma membranes, sustaining DNA structure, activating antioxidant mechanisms, enhancing osmolyte accumulation, and improving photosynthesis (Napieraj *et al*., 2023) [41].

During the plant's growth stages, germination is the first most important growth factor. Heat stress negatively affects several crops during seed germination, although the specific temperature ranges vary significantly among crop species (Haj Sghaier *et al.*, 2022) [57]. Rising temperatures during the germination and seedling stages are emerging as a notable concern, significantly impacting the production and productivity of Indian mustard in key mustard-growing regions (Rai *et al.*, 2020) [48]. The influence of heat stress extends across virtually all stages of plant growth, spanning from germination to maturity. It exerts an impact on plant development throughout its entire lifespan, albeit with significant variations in the threshold levels for heat stress at different developmental stages (Tushabe *et al.*, 2023) [60]. Decreased germination percentage, impaired plant emergence, production of abnormal seedlings, diminished seedling vigor, and reduced growth of radicles and plumules in germinated seedlings are prominent impacts attributed to heat stress across various cultivated plant species (Chaudhary *et al.*, 2022) [12]. In the present study, a decline in seed germination under heat stress conditions was observed. Notably, the most significant reduction in germination rates was observed in genotypes RH-1707 and RH-1708. There are several pieces of evidence that exogenous application of polyamines such as Put, spermidine and spermine protects plants against the damage caused by various types of abiotic stresses (Todorova *et al.*, 2015; Chen *et al.*, 2019) [15, 14]. Polyamines are the endogenous plant growth regulators, or they may act as intracellular messengers that encourage a number of physiological and biochemical processes in response to high temperature. As a result, they increase tolerance of plants to stresses by modifying growth and development (Kusano *et al.*, 2008; ElSayed *et al.*, 2022) [34, 20]. Earlier, Put was found to markedly improve the germination of seeds, indicating its potential as a mitigating agent against the adverse effects of heat stress on this vital stage of plant growth (Farooq *et al.*, 2008) [23]. In our study, we obtained similar results where Put was found to enhance seed germination.

Heat stress resulted in a decrease in the length of roots, shoots, as well as a reduction in leaf area, size and number. The decrease in root and shoot length suggests a compromised ability to explore soil for nutrients and water, as well as impaired above-ground growth (Mishra *et al.*, 2023) [38]. In the present study, heat stress significantly reduced various growth-related parameters such as shoot length, root length, fresh weight and dry weight. The most prominent decrease was shown by genotypes RH-1708, followed by RH-1707. The impact of exogenous application of Put shows an improvement in growth-related parameters which suggests its effectiveness in ameliorating heat-induced morphological damage. Our results were in agreement with the findings of El-Bassiouny *et al.* (2008) and Hassanein *et al.* (2013) [28] in wheat, Amooaghaie *et al.* in soybean (2011) [5] and Yadav *et al.* (2019) [66] in tomato.

In plants, exposure to climatic stress typically leads to a decrease in chlorophyll concentrations and a reduction in the photochemical reactions of thylakoid proteins. This decrease in chlorophyll pigments directly impacts the photosynthetic activity of crops (Sherin *et al.*, 2022) [52]. Temperature fluctuations have been reported to significantly influence photosynthesis in crops, primarily due to the diminished presence of chlorophyll. This indicates that changes in temperature can directly affect the plant's ability to photosynthesize efficiently, which is crucial for its growth and development (Muhammad *et al.*, 2021) [39]. The chlorophyll levels in plants depend on a delicate equilibrium between synthesis and degradation. When subjected to heat stress, this equilibrium is disrupted, resulting in reduced chlorophyll concentration in the plants (Hu *et al.*, 2020) [30]. In the present study, reduction in chlorophyll content across all four genotypes under heat stress conditions was observed. However, the most notable effect was observed in genotypes RH-1708. The exogenous application of Put was effective in enhancing chlorophyll levels in the plants. Several research have shown the beneficial roles of exogenous Put application on chlorophyll concentration in various crops under different unfavourable environments (Demetriou *et al.*, 2007; Shu *et al.*, 2012; Amin *et al.*, 2013; Jahan *et al.*, 2022) [16, 53, 4, 31]. Put may help stabilize the photosynthetic machinery within chloroplasts, preventing damage caused by various abiotic stresses (Shu *et al.*, 2015) [54]. Additionally, it has been shown to modulate the activity of various enzymes involved in chlorophyll biosynthesis and degradation pathways. Under heat stress conditions, Put may regulate these enzymatic processes to favor chlorophyll synthesis and inhibit its breakdown, ultimately leading to an increase in chlorophyll content (Jahan *et al.*, 2022) [31]. In our study, we found that the application of Put resulted in an increase in chlorophyll content across all genotypes studied, both in pots and field conditions. However, the most significant impact of Put was observed in the thermosensitive genotypes RH-1707 and RH-1708. This suggests that these genotypes exhibit higher responsiveness to Put treatment, leading to a more pronounced enhancement in chlorophyll content compared to other genotypes.

A key attribute of plants that exhibit enhanced tolerance and superior performance under heat stress is their RWC. Chlorophyll is known to be highly correlated with RWC of plants (Keyvan, 2010) [32]. This trait is considered crucial because it reflects the plant's ability to maintain hydration and physiological balance under stress conditions, particularly those induced by elevated temperatures. High RWC in plants typically indicates better water retention capabilities and effective cellular water management, which are vital for sustaining growth and metabolic activities under heat stress (Seleiman *et al.*, 2021) [50]. The genotypes RH-1707 and RH-1708 exhibited the most significant impacts, particularly during late sowing period under late heat stress. The decline in leaf RWC could be associated with reduced root quantity, mass, and growth in response to heat

stress conditions. This, in turn, may restrict the availability of water and nutrients to the aboveground parts of the plant (Banon *et al.*, 2004) [8]. The observed reduction in RWC may be attributed to the excessive loss of water through stomata during transpiration (Lobato *et al.*, 2008) [35]. Our findings align with research conducted on rice (Fahad *et al.*, 2016) [21] and wheat (Riaz *et al.*, 2021) [49]. Genotypes RH-1566 and RH-1999-42 showed superior water status maintenance under heat stress conditions. This resilience suggests that these genotypes possess inherent mechanisms or traits that enable them to efficiently manage water availability and mitigate the adverse effects of heat stress. The application of Put significantly boosted RWC across all examined genotypes. However, genotypes RH1707 and RH-1708 exhibited particularly remarkable enhancements in these parameters which underscore the potential of Put as a valuable intervention for enhancing water status in *B. juncea* under heat stress. This observation is consistent with the beneficial effects of Put application in improving water status previously reported in *Triticum aestivum* (Gupta *et al.*, 2003; Wasaya *et al.*, 2023) [26, 64] and in *Cabernet Sauvignon* (Zhao *et al.*, 2021) [68]. Put has been reported to stimulate root growth and development. By increasing root biomass and surface area, Put enhances the plant's capacity to absorb water from the soil, improving water uptake efficiency even under heat stress conditions (González-Hernández *et al.*, 2022) [25].

Heat stress decrease membrane stability and lead to excessive fluidity of membrane lipids, which is associated with the loss of physiological function. This membrane disruption hampers processes like photosynthesis that rely on the activity of membrane-bound electron transport enzymes (Prasertthai *et al.*, 2022) [45]. Heat stress also alter membrane composition and structure, leading to ion leakage. It is stated that the initial impact of heat on photosynthesis is more directly linked to membrane characteristics and the disruption of transfer mechanisms within chloroplasts than to a widespread denaturation of proteins (Hu *et al.*, 2020) [30]. The status of membrane integrity in plants under high-temperature is frequently determined by parameters like electrolyte leakage. Indeed, high-temperature stress is known for its detrimental impact on cell membrane integrity, resulting in an increase in electrolyte leakage from leaf tissue. Electrolyte leakage is widely recognized as a reliable indicator of heat stress, indicating the compromised integrity of cell membranes in such conditions (Wahid *et al.*, 2007) [62]. An increase in temperatures has been documented to disrupt cell membrane stability by inducing changes in the structure, lipid composition, and proteins of the membrane (Barghi *et al.* 2013) [9]. This disruption results in an elevation of electrolytes, indicating membrane injury, which is reported to be a direct consequence of high temperatures causing an increased production of highly toxic reactive oxygen species such as H_2O_2. Our investigation revealed a notable rise in membrane damage, evidenced by increased electrolyte leakage and lipid peroxidation. RH-1708 demonstrated increased membrane damage as indicated by the elevated levels of ions leakage and MDA suggesting a suggests a less efficient ROS neutralization mechanism. These observations are in line with the studies conducted by Rai *et al.* (2020) [48] and Ahmed *et al.* (2019) [2]. Additionally, it appears that genotypes RH-1566 and RH-1999-42 with higher tolerance, typically exhibit lower oxidative damage. This reduced stress response could be attributed to a more robust defense mechanism, encompassing antioxidant enzymes, osmoprotectants, stress-responsive genes, transcription factors, and integrated signalling pathways (Soengas *et al.*, 2018; Rai *et al.*, 2020) [56, 48]. Preserving membrane stability and integrity during stress conditions is a critical factor for plant tolerance, as it is essential for the sustained operation of photosynthetic and respiratory functions. This characteristic of maintaining membrane stability under heat stress likely contributes to the resistance of genotypes RH-1566 and RH-1999- 42, enabling them to exhibit tolerance. Polyamines being positively charged, have the ability to bind with the negatively charged phosphate groups in cell membranes. This interaction can lead to a decrease in membrane potential and a subsequent reduction in lipid peroxidation levels (Mathaba *et al.*, 2012) [37]. The exogenous application of Put significantly decreased ion leakage and lipid peroxidation under heat stress. This indicates the involvement of exogenous Put in mitigating heat-induced membrane damage and enhancing membrane stability (Wassie *et al.*, 2020) [65]. In this study, the application of Put notably diminished the membrane damage in both under heat stress, particularly in sensitive genotypes. Polyamines are essential in maintaining membrane integrity, as they help reduce electrolyte leakage and MDA levels. Thus, from overall results, it can be stated that heat stress have been observed to exert detrimental effects on the growth of *Brassica juncea*. However, application of putrescine (1mM) has shown notable enhancements in various parameters (Fig. 9).

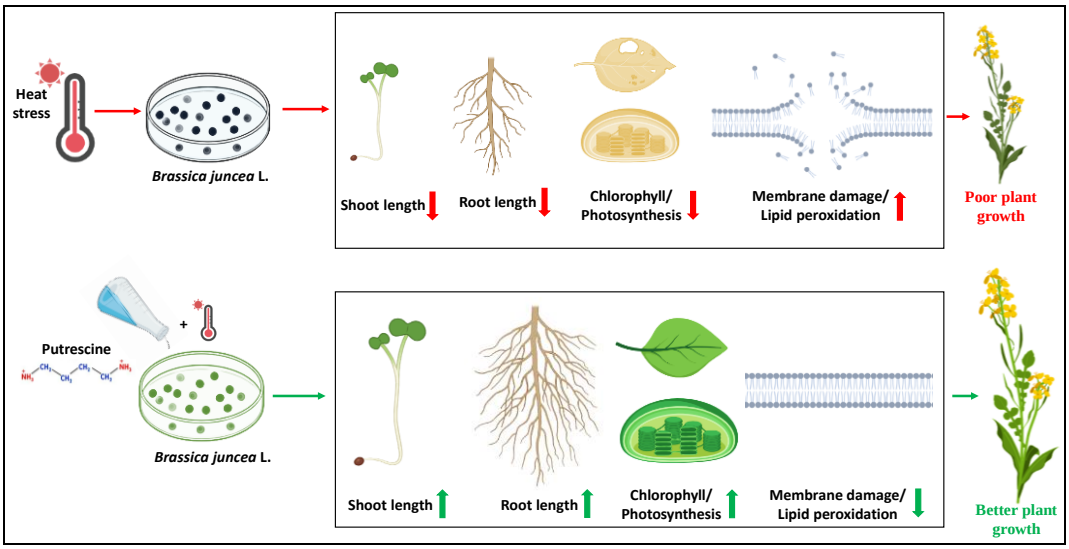

Fig 9: Effects of heat stress and Put application on *Brassica juncea* L. seedlings

Conclusion

The present investigation was conducted to assess the impact of heat stress on four *Brassica juncea* genotypes (RH-1707, RH-1708, RH-1566 and RH-1999-42) and explore the alleviating effects of exogenously applied Put. Heat stress reduces growth, including germination rates, root-shoot length, fresh and dry weight, chlorophyll content, RWC whereas increases electrolyte leakage and lipid peroxidation across all genotypes. The most pronounced effect of heat stress was observed in genotypes RH-1707 and RH-1708 whereas RH-1566 and RH-1999-42 performed better by maintain water status and membrane stability under heat stress. The application of Put showed a positive impact on enhancing growth parameters, chlorophyll content and RWC while reducing electrolyte leakage and lipid peroxidation in all genotypes under heat stress, but RH-1707 and RH-1708 showed more significant improvements. Thus, it can be concluded from the present investigation that application of Put (1 mM) promotes the growth of *Brassica juncea* under heat stress.

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