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Research Article

Optimizing the production of true shallot seed by inducing flowering in various shallot genotypes

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ABSTRACT

Shallot (Allium cepa var. aggregatum) productivity can be enhanced through various methods, including using true shallot seed (TSS). Successful shallot breeding requires flowering to improve traits through gene transfer between genotypes and to produce TSS. However, one of the primary challenges in TSS production is the variability in the flowering ability of different varieties. This study aimed to examine the impact of flowering induction on flowering traits, pollen viability, and TSS production in several shallot genotypes. The study employed a randomized complete block design with two factors: shallot genotypes and induction treatments. The study found that vernalization treatment significantly increased the percentage of flowering plants in the Bauji, Bima Brebes, and Tajuk genotypes. Additionally, the combination of vernalization (V) and gibberellin (GA_3) (V+G) successfully induced flowering in the Maja Cipanas genotype compared to the control. The Bima Brebes genotype exhibited the highest pollen viability following both vernalization and V+G treatment. Furthermore, vernalization also led to an increase in TSS weight in shallots. These findings suggest that vernalization could be an effective strategy for enhancing TSS production in highland areas, thereby supporting the development of high-yielding shallot varieties. Significant positive correlations were observed between TSS weight and several traits, including the percentage of flowering plants, umbel number, umbel diameter, flower number, pollen viability, percentage of plants producing TSS, capsule number, and TSS number.

Keywords: *Allium cepa*; correlation; gibberellin; varieties; vernalization

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INTRODUCTION

The shallot (*Allium cepa* var. *aggregatum*) is widely cultivated and utilized across Indonesia and on a global scale. Batu Ijo, Super Philip, Bima Brebes, and Rubaru varieties are recognized for their savory flavor and robust aromas, establishing them as raw materials for fried shallots (Yofananda et al., 2021). Beyond their culinary utility, the shallot is posited to convey substantial health benefits. Recent studies have indicated that shallot extract has the potential to serve as a traditional remedy for hair loss, attributed to the presence of phenolic compounds with anti-inflammatory properties (Ruksiriwanich et al., 2022). Furthermore, shallot extract has demonstrated antiallergic activity, which can help alleviate symptoms of allergic rhinitis or hay fever (Arpornchayanon et al., 2022).

The rising consumption of shYofananallots necessitates sustained efforts to meet domestic demand. True shallot seed (TSS) is one way to enhance shallot yield. Farmers commonly use bulbs in Indonesia for planting due to their ease of propagation, planting, and shorter harvest time. However, the continuous use of bulbs can lead to the transmission of systemic disease and hinder the improvement of plant traits, potentially resulting in decreased shallot production. According to Pangestuti and Sulistyaningsih (2011), TSS proffers numerous advantages over bulb-derived seeds, such as diminished seed requirements, lowered costs of seed production, decreased storage space necessities, extended seed life, and simplified distribution, which is both more cost-effective and confers a reduced risk of virus or disease transmission. The limited storage and dormancy periods of tubers represent significant constraints, resulting in tubers needing to be available in adequate quantities and at the appropriate developmental stages during the growing season (Rosliani et al., 2021). Despite TSS potentially reducing the dependency on pesticides and fertilizers, the production of TSS presents a higher risk than the conventional bulb methodologies (Adiyoga, 2023). Risks accompanying TSS include the demand for more sophisticated farming practices, the longer it takes to produce mini tubers, and the scarcity of TSS seeds. While both bulbs and TSS have advantages and disadvantages, TSS offers more significant benefits in enhancing shallot productivity.

Currently, shallot cultivation from TSS has yet to be widely adopted by farmers in Indonesia. The TSS cultivation is primarily due to the limited availability of TSS and the challenges farmers face in growing shallots from seeds. Consequently, the private sector has broadly undertaken TSS development and propagation (Manwan et al., 2020). One of the significant challenges in enhancing TSS production is the variability in flowering ability among different shallot genotypes. Shallot genotypes exhibit diverse patterns of flowering ability; some can produce flowers and seeds, while others cannot (Marlin, 2018). The flowering capability of shallot is influenced by several factors, including agro-climatic conditions and the genotypic characteristics of the plant (Irawan et al., 2021).

The induction of flowering and seed formation in shallot can be facilitated through vernalization or applying growth regulators such as gibberellin (GA_3). Vernalization allows plants to acclimate to local climatic conditions, thereby influencing the optimal timing of flowering (Kim et al., 2009). This process involves the exposure of plants to cold temperatures for a specific duration, thereby enhancing their flowering potential (Song et al., 2019). Gibberellin plays a significant role in regulating flowering in *Arabidopsis thaliana* by interacting with various genetic pathways involved in the flowering process (Bao et al., 2020). The GA_3 signaling pathway is an additional regulatory factor, predominantly influencing flowering under long-day conditions (Dong et al., 2017).

The most effective and efficient method for GA_3 application to enhance flowering and TSS yield was soaking the seedling bulbs for 30 minutes in a gibberellin solution at a concentration of 200 ppm (Sumarni et al., 2013). The combination of vernalization and gibberellin at 200 ppm for 30 minutes significantly increased the flowering percentage, the number of umbels per plant, and the number of flowers per umbel in the highland (Fahrianty et al., 2020). The application of vernalization at 10 ^{0}C for four weeks (Rosliani et al., 2018) and immersion in GA_3 at concentrations of 100-200 ppm (Fahrianty et al., 2020; Sumarni et al., 2013) has been shown to enhance flowering in shallots. Farmers widely use the Bima Brebes variety, and it has demonstrated the ability to flower across various regions. Meanwhile, Maja Cipanas, Batu Ijo, SS Sakato, Tajuk, and Bauji are commercial varieties valued for their adaptability and disease resistance. However, their flowering ability has yet to be extensively studied and still needs to be explored. This study aimed to examine the impact of vernalization and the combined application of vernalization and gibberellin on flowering percentage, pollen viability, and TSS weight in several genotypes.

MATERIALS AND METHODS

Plant material and experimental design

This research was conducted from August to December 2023 at the Pasir Sarongge Experimental Farm, Cianjur (1,100 meters above sea level). The study employed a two-factor Randomized Complete Block Design (RCBD). The first factor was shallot genotypes, with six levels: Bima Brebes, Maja Cipanas, Batu Ijo, Bauji, Tajuk, and SS Sakato. The second factor comprised flowering induction treatments with three levels, including vernalization (V), vernalization followed by gibberellin soaking (V+G), and control with no treatment (C). This design resulted in 18 combinations, each replicated three times, resulting in 54 experimental units. Each experimental unit consisted of five polybags arranged in a single row, each containing two plants, totaling ten plants per unit. Five plants were randomly selected from each unit for sampling.

Flowering induction treatment

Flowering induction treatments included vernalization (V), vernalization combined with gibberellin (V+G), and a control (C). For the vernalization treatment, shallot bulbs were stored at 10 ^{0}C for 30 days in cold storage (Rosliani et al., 2018). Following this period, the bulbs were held at room temperature for one day before planting. In the V+G treatment, the vernalized bulbs were subsequently immersed in a gibberellin solution at a concentration of 200 ppm for 30 minutes (Sumarni et al., 2013; Fahrianty et al., 2020). The control bulbs were not subjected to vernalization or gibberellin treatment.

Planting and maintenance

Shallot planting was carried out in an open field using polybags filled with a planting medium of husk charcoal, soil, and manure in a 1:1:1, v:v:v. At planting, a base fertilizer of SP-36 was applied at 1 g per polybag. Mutiara NPK fertilizer (16:16:16) was applied at a dosage of 0.735 g per polybag at ten-day intervals since the bulbs were planted. Boron was applied via foliar spraying at 3, 5, and 7 weeks after planting (WAP), using a recommended dose of 3 kg ha^{-1} (Rosliani et al., 2012). Pollination was carried out by *Apis cerana* (Palupi et al., 2015) by introducing the insect into the experimental plots, which were constructed using insect nets and bamboo after the umbel spathes had split. A sugar solution was provided weekly as a food source of *A. cerana* throughout the flowering period. Harvesting occurred when the shallot capsules turned yellowish-brown, and some had split to reveal black seeds.

Pollen viability assay

Pollen viability was assessed using the in vitro pollen germination method, as described by Brewbaker and Kwack (1963). The germination medium was prepared with 10 g sucrose, 0.010 g boric acid, 0.030 g calcium nitrate tetrahydrate, 0.020 g magnesium sulfate heptahydrate, 0.010 g potassium nitrate, and 100 ml distilled water. Flower samples were collected at the half-bud stage. Pollen grains were placed on a slide, and 2-3 drops of the germination medium were added. The slides were then incubated in a petri dish for 24 hours. Observations were made using an Olympus CX23 microscope at 10x magnification. Germinated pollen was identified by a pollen tube emerging from the pollen grain. The number of germinated pollen grains was counted by examining three randomly selected points of view per sample.

Observation characters

The components of shallot vegetative growth characteristics were observed, including plant height (cm), leaf number, and leaf diameter (mm), measured 35 days after planting (DAP) when the majority of shallots reached optimum vegetative growth. Flowering characteristics were evaluated starting from 27 DAP, when umbels first appeared, and included the percentage of flowering plants (%), umbel number per plant, umbel diameter (mm), and flower number per umbel. Pollen viability was assessed by calculating the number of pollen grains that developed into pollen tubes and comparing it

to the total number of pollen grains. Component of true shallot seed (TSS) characteristics, including the percentage of plants producing TSS (%), capsule number per plant (fruit), TSS number per plant (seed), and TSS weight per plant (g), were measured at harvest at 100 DAP.

Data analysis

Data on vegetative growth, flowering, pollen viability, and TSS production were analyzed using analysis of variance (ANOVA) to evaluate the effects of individual factors and their interactions. Treatments that showed significant effects were further analyzed using the honestly significant difference (HSD) test at the 5% significance level. Statistical analyses were conducted with SAS OnDemand for Academics (https://welcome.oda.sas.com) and STAR 2.0.1 software. Relationships among the observed traits were examined through correlation analysis. Correlation analysis was performed using R version 4.4.1 with the 'agricolae' package.

RESULTS AND DISCUSSION

Vegetative growth of shallot

The result of ANOVA revealed that both genotype and induction treatment factors had significant effects (P<0.01) on plant height, leaf number, and leaf diameter, while the interaction between these factors was not significant (Table 1). The Batu Ijo (46.10 cm), Maja Cipanas (44.60 cm), and Bima Brebes (42.79 cm) exhibited the highest average plant heights among the genotypes. Regarding the leaf number, the Batu Ijo genotype also had the highest average (36.5), similar to the SS Sakato genotype (29.7). The largest leaf diameter was observed in the Maja Cipanas (7.85 mm) and Batu Ijo (7.32 mm) genotypes.

The Batu Ijo genotype demonstrated superior phenotypes across all three observed vegetative traits. Genotypes with robust vegetative growth can efficiently utilize nitrogen, which is crucial for amino acid synthesis, protein formation, and other vital metabolic processes driving plant growth (Abdissa et al., 2011). The Batu Ijo exhibited a superior ability to adapt to highland conditions compared to other genotypes, as it can perform well across seasonal variations and altitude gradients, including in both rainy and dry seasons and lowland and highland environments (Rachmawati et al., 2023).

Table 1. Vegetative traits of six shallot genotypes from induction treatments.

Treatment	Plant height (cm)	Leaf number	Leaf diameter (mm)
	Genotypes		
Bauji	37.51cd	27.6b	4.81c
Bima Brebes	42.79ab	27.7b	5.78b
Batu Ijo	46.10a	36.5a	7.32a
Maja Cipanas	44.60ab	24.3b	7.85a
Tajuk	35.26d	26.7b	5.01bc
SS Sakato	40.69bc	29.7ab	4.96bc
	Induction treatments		
Control	43.82a	35.7a	6.41a
Vernalization	40.12b	25.7b	5.79b
Vernalization + GA_3	39.53b	24.9b	5.67b
Interaction (F-test)	ns	ns	ns

Note: Values followed by the same letter within the same column are not significantly different according to the HSD test at the 5% level, ns = not significant.

Table 1 shows that the highest plant height was observed in bulbs that did not receive any pre-planting treatment (control), with an average height of 43.82 cm. The control treatment also recorded the highest leaf number, averaging 35.7, and exhibited the largest leaf diameter (6.41 mm) compared to the other treatments. A previous study reported that vernalization at 10 °C optimally enhances vegetative growth, particularly regarding plant height (Nawfetrias et al., 2023). In contrast, the present study found a

decrease in plant height, leaf number, and leaf diameter with vernalization treatment compared to the control. This discrepancy appears to be attributed to applying vernalization at a temperature of 10 °C, which may not be suitable for the genetic characteristics of each tested genotype. Each plant genotype exhibited varying capacities to tolerate cold stress. Low temperatures that do not align with the physiological requirements of the genotype can hinder vegetative growth, including the number of leaves in garlic (Wu et al., 2016). Plants employ protective mechanisms against low temperatures by reducing vegetative growth and enhancing antioxidant enzyme activity, which can disrupt cellular functions and lead to tissue necrosis (Wu et al., 2016). Furthermore, cold temperatures can adversely affect the physiological functions of chloroplasts, resulting in decreased photosynthetic efficiency (Liu et al., 2018).

The application of GA_3 resulted in a reduction in all vegetative traits compared to other treatments. This finding aligns with the studies by Marlin et al. (2021) and Nawfetrias et al. (2023), which report that shallots that are not treated with gibberellin exhibit taller plant growth compared to those treated with concentrations of 150-200 ppm. In contrast, Oktaviani et al. (2020) observed no significant difference in plant height between 0 ppm and 200 ppm gibberellin concentrations at 35 DAP, although the highest average plant height was achieved with 0 ppm. Each genotype exhibits varying tolerance to the applied gibberellin concentration and soaking duration. Adding gibberellin to bulbs that had undergone vernalization did not improve plant growth compared to the control. This may be due to the 200 ppm gibberellin concentration and 30-minute soaking duration being unsuitable for specific genotypes, inhibiting vegetative growth. Gibberellin is likely more effective and optimal in promoting plant growth under long-day conditions (Dong et al., 2017). Rostami and Mohammadi (2022) also noted that timing, dosage, application methods, and plant species influence the effects of GA_3.

Shallot flowering

The analysis of variance revealed significant differences for both main factors and interactions between shallot genotypes and flowering induction treatments ($P<0.01$). The Bima Brebes genotype exhibited the highest percentage of flowering plants (45.56%), with all three treatments effectively inducing flowering (Table 2). The Bima Brebes (26.67%), Bauji (10.00%), and Tajuk (10.00%) genotypes showed natural flowering, although at lower percentages compared to those induced by treatments. This response may be influenced by the lower temperatures characteristic of the highland area of Sarongge, where 16-18 °C is considered optimal for shallot flowering (Rosliani et al., 2018; Hantari et al., 2020). The SS Sakato genotype did not produce flowers in the highland area of Sarongge, irrespective of the treatment applied, including control conditions, vernalization (V), or the combination of vernalization and gibberellin (V+G). Although saccording to the variety description, SS Sakato is adaptable to highland conditions, particularly in the Solok Regency, it failed to flower in Sarongge highland. In contrast, Bima Brebes, described as more suited to lowland conditions, could grow and flower in highlands. . This observation suggests that genetic factors significantly influence flowering ability. The gene shLFY, which plays a crucial role in flowering capability, is expressed more abundantly in the Bima Brebes genotype than in other varieties (Irawan et al., 2021).

Table 2. The percentage of flowering plants of six shallot genotypes from induction treatments.

Induction treatments	Percentage of flowering plants (%)						Average
	Bauji	Bima Brebes	Batu Ijo	Maja Cipanas	SS Sakato	Tajuk	
Control	10.00c	26.67c	0.00a	0.00b	0.00a	10.00b	7.78c
Vernalization	43.33a	63.33a	13.33a	26.67a	0.00a	26.67a	28.89a
Vernalization + GA_3	26.67b	46.67b	10.00a	26.67a	0.00a	0.00b	18.33b
Average	26.67b	45.56a	7.78de	17.78bc	0.00e	12.22cd	

Note: Values followed by the same letter within the same column is not significantly different according to the HSD test at the 5% level.

The increase in flowering percentage observed in specific genotypes was achieved by applying exogenous flowering induction treatments (Table 2). The interaction between the Bauji, Bima Brebes, and Tajuk genotypes with the vernalization treatment resulted in a significant increase in flowering percentage. This finding suggests that vernalization at 10 °C for 30 days effectively induces and enhances flowering in these three genotypes. Consistent with these results, Siswadi et al. (2020) also reported that vernalization significantly increases the flowering percentage in shallots. Shallot flowering can be induced by cold storage treatments, with an optimal temperature range of 5-10 °C, while higher temperatures delay the development of inflorescences (Krontal et al., 2000). Cold storage for several weeks is sufficient to induce flowering, although extended periods may further accelerate the flowering process to its optimal point (Trevaskis et al., 2007). Vernalization induces flowering only in specific genotypes due to genetic differences that influence the plant's physiological response to low temperatures. Each genotype exhibits varying adaptability to environmental conditions, including cold temperatures. Some genotypes possess a flowering gene, such as gen shLFY, more responsive to vernalization, thereby triggering the internal mechanisms required to initiate flowering after exposure to low temperatures. In contrast, plants with less responsive genetics do not experience significant changes, even when subjected to vernalization treatment (Amasino, 2010).

Adding gibberellin at a concentration of 200 ppm for a 30-minute soaking duration in vernalized bulbs did not induce flowering in the Tajuk genotype (Table 2). Each genotype requires different vernalization conditions in terms of temperature and duration. For the Tajuk genotype, applying gibberellin at a concentration of 200 ppm for 30 minutes was ineffective, as it did not induce flowering. A noticeable physical change occurred during the soaking process, with the bulbs exhibiting signs of wrinkling. Furthermore, gibberellin application did not increase the flowering percentage in the Bauji and Bima Brebes genotypes compared to vernalization. The Batu Ijo and Maja Cipanas genotypes showed similar responses, with no significant differences in flowering percentage between vernalization (V) and gibberellin application (V+G). Previous research has indicated that GA_3 application does not increase the percentage of flowering plants per plot (Triharyanto et al., 2018). Since most plants, including shallots, naturally contain gibberellin hormones in an inactive and limited state, the effects of GA_3 application can vary depending on the specific needs of each genotype (Triharyanto et al., 2018; Kasim et al., 2020).

The five genotypes that successfully flowered were further evaluated for flowering traits. The interaction between genotypes and induction treatments significantly affected all flowering traits ($P<0.01$), as detailed in Table 3. The observed responses varied among the genotypes. Each genotype responds to flowering induction. In the Bauji genotype, the number of flowers was reduced by adding vernalization and combining vernalization with GA3. This may be due to the temperature and duration of vernalization or an inappropriate gibberellin concentration and soaking duration, which inhibited flower formation in this genotype. Conversely, the interaction between the Bima Brebes genotype and vernalization resulted in the highest average umbel diameter. Marlin et al. (2021) reported that vernalization can increase umbel diameter in shallots. Larger umbel diameters generally lead to more capsules per umbel, potentially resulting in more seeds per umbel (Purnamasari et al., 2023). Although the average flower number per umbel in the Bima Brebes genotype was higher with vernalization, this difference was insignificant compared to other treatments. Vernalization aids in converting complex sugars in the bulbs to simpler sugars, which are more readily available for the transition to the generative phase, thus increasing the number of flowers (Yalamalle, 2016).

Table 3. Interaction between five shallot genotypes and induction treatments on flowering traits.

Characters	Induction treatments	Genotypes				
		Bauji	Bima Brebes	Batu Ijo	Maja Cipanas	Tajuk
Umbel number per plant	C	1.00a	1.33b	0.00b	0.00b	1.00a
	V	1.27a	2.26a	1.00a	1.00a	1.33a
	V+G	1.22a	2.33a	1.00a	1.00a	0.00b
Umbel diameter (mm)	C	36.23a	39.07b	0.00c	0.00b	29.17a
	V	35.33ab	52.53a	34.67b	31.57a	32.33a
	V+G	30.57b	40.83b	42.17a	33.33a	0.00b
Flower number per umbel	C	72.33a	82.33a	0.00c	0.00b	50.33a
	V	44.00b	103.00a	55.33b	58.33a	58.33a
	V+G	40.67b	85.67a	84.00a	80.33a	0.00b

Note: Value followed by the same letter within the same column is not significantly different according to the HSD test at the 5% level. C = control, V = vernalization, V+G = vernalization combined with gibberellin.

Adding gibberellin (V+G) to the Batu Ijo genotype increased average values for umbel diameter and flower number. Gibberellin exhibits effects similar to vernalization, as low-temperature treatments can enhance GA_3 levels at the shoot tips (Song et al., 2019). In contrast, no significant interaction was observed between the Maja Cipanas genotype and vernalization (V) or GA_3 addition (V+G). Similarly, the Tajuk genotype did not show significant interactions with the control (C) or vernalization (V) treatments.

Pollen viability and true shallot seed production

Table 4 shows that the two factors interaction was significant (P<0.01). Both vernalization (V) and vernalization with gibberellin (V+G) significantly enhanced pollen viability in the Bima Brebes compared to the control. These results confirm that both vernalization (V) and gibberellin (V+G) applications effectively improve pollen viability in this genotype. For the Bauji, Batu Ijo, and Tajuk genotypes, no significant differences were observed among the treatments concerning their effects on flowering stimulation. In contrast, the Maja Cipanas genotype exhibited the highest pollen viability percentage with adding GA_3 compared to vernalization. This result indicates that GA_3 application improves pollen viability in the Maja Cipanas genotype more effectively than only vernalization. High pollen viability enhances the potential for successful pollination, ultimately contributing to the formation and increase in the number of capsules and TSS per plant.

Table 4. Interaction between genotypes and induction treatments on pollen viability of shallots.

Induction treatments	Percentage of pollen viability (%)				
	Bauji	Bima Brebes	Batu Ijo	Maja Cipanas	Tajuk
Control	27.22a	34.18b	0.00b	0.00c	12.51a
Vernalization	27.18a	41.85a	14.85a	14.83b	15.08a
Vernalization + GA_3	24.36a	41.77a	11.43a	23.07a	0.00b

Note: Value followed by the same letter within the same column is not significantly different according to the HSD test at the 5% level.

The interaction between genotype and induction treatments significantly affected the percentage of plants producing TSS, capsule number, and TSS number (P<0.01). However, it did not significantly affect TSS weight (Table 5). For the Bauji genotype, there was a significant interaction in capsule number , with the addition of gibberellin (V+G) leading to a decrease in the average capsule number compared to the control and vernalization treatments. The vernalization treatment effectively increased the percentage of plants with TSS, capsule number, and TSS number in the Bima Brebes genotype. High pollen viability enhances the potential for successful pollination, ultimately contributing to the formation and increase in the capsule number and TSS number per plant.

Table 5. TSS production of five shallot genotypes from induction treatments.

Characters	Induction treatments	Genotypes					Average
		Bauji	Bima Brebes	Batu Ijo	Maja Cipanas	Tajuk	
The percentage of plants producing TSS (%)	C	10.00a	13.33b	0.00b	0.00b	10.00ab	6.67b
	V	10.00a	30.00a	10.00ab	16.67a	13.33a	16.00a
	V+G	10.00a	23.33ab	13.33a	13.33a	0.00b	12.00a
	Average	10.00b	22.22a	7.78b	10.00b	7.78b	
Capsule number per plant (fruit)	C	24.0a	40.0b	0.0b	0.0b	17.0a	16.2b
	V	19.0ab	60.7a	31.3a	15.7b	25.7a	30.5a
	V+G	7.7b	51.0ab	26.3a	38.3a	0.0b	24.7a
	Average	16.9b	50.6a	19.2b	18.0b	14.2b	
TSS number per plant (seed)	C	37.3a	69.0b	0.0a	0.0b	34.3ab	28.3b
	V	32.0a	130.3a	32.3a	18.3ab	38.0a	50.2a
	V+G	21.7a	106.0ab	30.0a	47.7a	0.0b	41.1b
	Average	30.3b	101.8a	20.8b	22.0b	24.1b	
TSS weight per plant (g)	C	0.08	0.22	0.00	0.00	0.04	0.07b
	V	0.06	0.39	0.07	0.03	0.06	0.12a
	V+G	0.06	0.30	0.06	0.08	0.00	0.10b
	Average	0.06b	0.30a	0.04b	0.04b	0.03b	

Note: Value followed by the same letter within the same column and row is not significantly different according to the HSD test at the 5% level. C = control, V = vernalization, V+G = vernalization combined with gibberellin.

The control treatment did not initiate flowering in the Batu Ijo and Maja Cipanas genotypes, leading to no significant differences in the observed traits of Batu Ijo (Table 5). The V+G treatment increased the number of capsules in the Maja Cipanas genotype, and the highest average number of TSS in Maja Cipanas was also obtained with the V+G treatment. However, it did not significantly differ from the vernalization (V) treatment. In the Tajuk genotype, vernalization enhanced the percentage of plants producing TSS and the number of TSS. However, these results did not differ significantly from those obtained with the V+G treatment. Previous studies have indicated that vernalization can improve the number of seeds per umbel in onion (Yalamalle, 2016) and garlic (Kaur & Dhall, 2017).

A significant effect on TSS weight was observed for single factors, specifically genotype and flowering induction factor (Table 5). The highest TSS weight was obtained from the Bima Brebes genotype, with a weight of 0.30 g. Meanwhile, the vernalization treatment was effective in increasing TSS weight compared to other treatments. TSS weight is not always affected by the number of flowers per umbel because not all capsules on the umbel contain seeds (Purnamasari et al., 2023). Empty capsules can occur due to limited pollen adhesion to the stigma, resulting in a limited number of seeds developing (Palupi et al., 2015). Capsule or seed formation depends on the activity of pollinating insects in pollinating the shallot flowers. Additionally, high pollen viability can lead to a greater seed set and, consequently, increased TSS production when combined with movement of active pollinators, as indicated by the number of capsule per plant. A high number of seeds does not always correspond to high TSS weight because some seeds may be non-viable.

Correlation analysis

TSS production is a challenge and a critical aspect of shallot breeding. The primary trait observed for TSS is TSS weight per plant. Efforts to increase TSS weight through genetic improvement or selection are expected to enhance seed availability across different seasons. The correlation between these two traits reflects the proportion of variance due to genetic factors, particularly the effects of pleiotropic genes. Understanding trait correlations is essential for developing effective selection indices in plant breeding programs (Sharma et al., 2021).

The research results indicated that TSS weight per plant was not significantly correlated with vegetative growth traits (Figure 1). This research suggests that efforts to

enhance TSS production can be pursued without affecting the plant's vegetative growth, particularly plant height, leaf number, and leaf diameter. The assimilates produced during the vegetative growth phase are more influential for bulb formation than seed production (Dianawati & Yulyatin, 2019). Additionally, initiating flowering requires appropriate temperature and day length, while TSS formation depends on the assistance of pollinating insects, which play a crucial role in efficiently transferring pollen.

This study revealed positive and significant correlations between TSS weight and several traits: percentage of flowering plants (r = 0.75), umbel number (r = 0.90), umbel diameter (r = 0.85), flower number (r = 0.79), percentage of pollen viability (r = 0.87), percentage of plants producing TSS (r = 0.87), capsule number (r = 0.91), and TSS number (r = 0.99). These results suggest that increasing the percentage of flowering plants, umbel number, umbel diameter, flower number, percentage of pollen viability, percentage of plants producing TSS, capsule number, and TSS number contributes to higher TSS weight. This finding is consistent with research by Rosliani et al. (2014) and Dianawati and Yulyatin (2019), which indicates that a high number of capsules correlates with the TSS number and TSS weights.

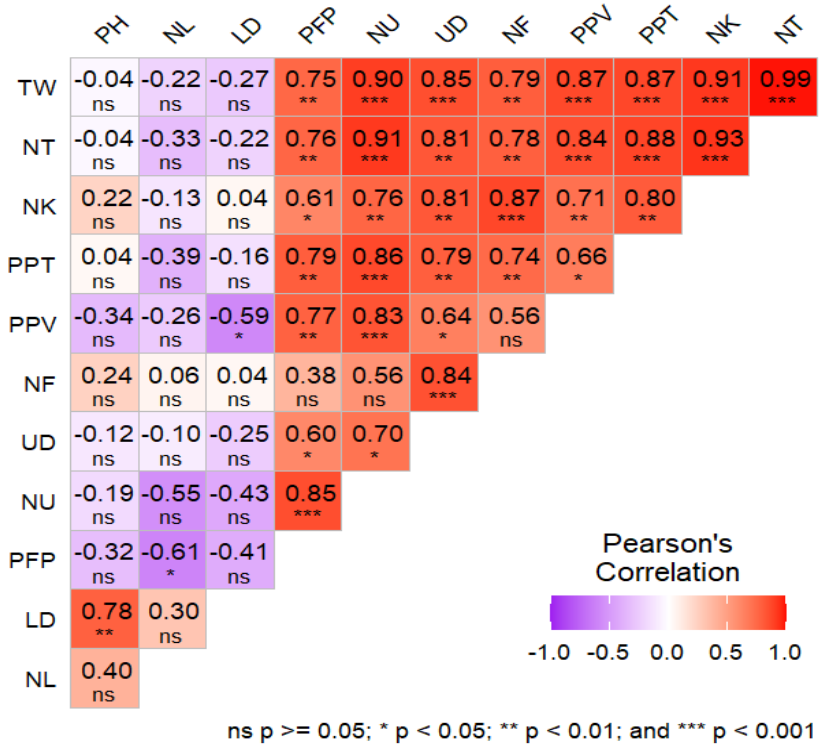

Figure 1. Correlation coefficients among agronomic traits from 12 treatment combinations. TW = TSS weight, NT = TSS number, NK = capsule number, PPT = percentage of plants producing TSS, PPV = percentage of pollen viability, NF = flower number, UD = umbel diameter, NU = umbel number, PFP = percentage of flowering plants, LD = leaf diameter, NL = leaf number, PH = plant height.

CONCLUSIONS

Vernalization treatment significantly increased the flowering percentage of the Bauji, Bima Brebes, and Tajuk genotypes. Additionally, the combination of vernalization and gibberellin (GA_3) effectively induced flowering in the Batu Ijo and Maja Cipanas genotypes compared to the control. The highest pollen viability was observed in the Bima Brebes genotype under both vernalization (V) and the combined vernalization and GA_3 (V+G) treatments. Furthermore, vernalization enhanced TSS weight in shallots. These results suggest that induction treatments, especially the application of vernalization, could be

strategically employed to improve the quantity of TSS production in highlands, thereby supporting the development of high-performing shallot varieties.

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