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SHALLOT (*ALLIUM CEPA* VAR. *AGGREGATUM*) GENOTYPES AND THEIR CROSSBREDS RESISTANCE TO FUSARIUM WILT DISEASE

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SUMMARY

Shallots are valuable horticultural products consumed worldwide that offer numerous health benefits. Fusarium wilt disease severely limits shallot production, reducing bulb yield by up to 50%; furthermore, wilt disease-resistant shallot cultivars are insufficient. This study confirmed that the 'Batu Ijo' cultivar is resistant to Fusarium wilt, with the lowest percentage of affected bulbs (29.5%). In contrast, the cultivar 'Bima Brebes' showed the highest incidence of Fusarium infection (41.6%). Neither 'Batu Ijo' nor 'Katumi' became resistant parents when crossbred with susceptible plants because both failed to bloom until the end of the plant generative stage. Thus, to generate diverse segregating populations, a cross combination derived from 'Biru Lancor' (resistant parent) and 'Bima Brebes' ensued. The results showed lower values for the number of mature capsules, true seed shallot weight per plant, and seedling vigor were evident in the progenies than in the parental genotypes. These poor crossing results were due to several factors, including an inappropriate photoperiod for the crossbreeding of 'Bima Brebes' and 'Biru Lancor.' Out of 30, 17 plants showed symptoms of Fusarium wilt disease. Meanwhile, the 13 resistant individuals exhibited similarities with the male parent 'Biru Lancor' during ISSR molecular analysis, suggesting that the male parent may play a role in transmitting disease resistance. The selection of parental individuals and crossbreeding factors are crucial for successful shallot crossbreeding. This study bears significant implications for further development of shallot crossbreeding, potentially leading to new disease-resistant cultivars.

Keywords: Shallot (*Allium cepa* var. *aggregatum*), Fusarium wilt resistance, cross breeding, self-compatibility, ISSR markers, inheritance

Key findings: Flower-based research plays a vital role in shallot breeding. Fusarium wilt resistance in offspring populations can be a basis for developing new resistant shallot cultivars.

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INTRODUCTION

Shallots (*Allium cepa* var. *aggregatum*) are essential bulb vegetables within the Allium family, consumed worldwide for their health benefits. Furthermore, with high preferences in French and Asian cooking, make them as gourmet onions, which are delicious either raw or cooked and have a wide range of end uses. Shallots have a global consumption. Society's increasing consumption needs the critical enhancement of Shallot's global production. Shallots also benefit agriculture as plant growth regulators, as the auxin content of shallot bulbs can induce root and stem cell elongation (Gresiyanti *et al.,* 2021).

The Fusarium wilt disease caused by *Fusarium oxysporum* is a foremost threat to shallot production. In particular, percent incidence and yield losses due to the Fusarium wilt disease in shallot crops increase with a predicted continuing trend (Wibowo *et al.,* 2023). The Fusarium wilt can reduce bulb yield by up to 50% (Hadiwiyono *et al.,* 2020). Additionally, a previous study reported a 45% loss in shallot bulb yield in the field due to a Fusarium wilt incidence, with an additional 10%-12% loss in storage (Sintayehu *et al.,* 2011). Several *F. oxysporum* races' testing on shallot samples has prevailed, which showed more than 50% aggressive reactions on shallot plants and even caused seedling death in Finland (Haapalainen *et al.,* 2016). *Fusarium oxysporum* is a systemic fungus that is difficult to control using fungicides; therefore, enhancing genetic diversity for resistance to Fusarium wilt disease among shallots is crucial in developing resistant cultivars.

Successful development of shallot cultivars resistant to Fusarium wilt disease remains relatively limited. Reports of some shallot cultivars, such as 'Tiron' and 'Kuning,' as resistant to Fusarium wilt disease have come out in Indonesia (Wiyatiningsih *et al.,* 2009). Similarly, among six shallot cultivars tested for resistance against Fusarium wilt, Prakoso *et al.* (2016) reported 'Batu Ijo' as moderately resistant. Furthermore, in a recent study conducted by Aprilia *et al.* (2018) on 19 shallot cultivars tested for Fusarium wilt responses, only two (Batu Ijo and Rubaru) reached categories as resistant to the disease.

Genetic diversity is achievable by crossbreeding shallot genotypes resistant or susceptible to Fusarium wilt disease. Indeed, improvements in shallot populations have progressed through 11 clones' crossbreeding to obtain a wide-genetic-variability germplasm collection in search of resistance to Fusarium wilt (Putrasamedja, 2010). As desired plant traits can be transmittable to the new generation through crossbreeding, crossbreeding among Fusarium-resistant and susceptible shallot genotypes could produce new resistant genotypes magnified by developing new gene combinations with specific functions (Wulf and Moscou, 2014). Therefore, populations obtained through crossbreeding need more screening for resistance-related traits inherited from the parental genotypes, and the emerging genetic diversity must require analysis using molecular markers to identify genes responsible for the resistance. Detecting parental strains with dominant or recessive alleles and individuals with genetic variations can proceed using molecular markers (Azrai, 2005). Specifically, Inter-simple sequence repeat (ISSR) markers based on PCR can safely help amplify two short nucleotides.

Herein, growing a shallot cross-population to obtain botanical seed, also known as a true shallot seed (TSS), is one of the best strategic solutions to improve production and yield in shallot breeding programs (Manwan *et al.,* 2020). The variability within TSS received further analysis and definition according to the diversity of Fusarium wilt disease resistance-related traits. Therefore, the study objective was to investigate the patterns of inheritance of disease resistance-related traits in hybrid shallot progenies for their consideration while evaluating plant disease resistance.

MATERIALS AND METHODS

Six shallot cultivars, namely, 'Batu Ijo,' 'Bima Brebes,' 'Kuning,' 'Maja Cipanas,' 'Biru Lancor,' and 'Katumi,' served as plant genetic materials in a study conducted between May 2022 and January 2023. The initial planting of shallot genetic material commenced at the IPB Experimental Garden, Pasir Sarongge, Cianjur, West Java (1200 masl), and molecular analyses ran at the Tajur Experimental Garden and Center for Tropical Horticulture Studies Laboratory, Bogor, West Java, Indonesia.

Analysis of shallot genotypes' resistance to fusarium wilt

The study began with the genetic material's planting for inoculation with the disease following standard cultivation practices for shallots in polybags (Hawayanti *et al.,* 2021). The *F. oxysporum* inoculum's propagation on a potato dextrose agar (PDA) medium included 0.125 g/L streptomycin and incubated for five days (Cahyaningrum *et al.,* 2020). Seven days after planting, shallot seedlings sustained *Fusarium* sp. Inoculation in the growing medium using the drenching method (Wijoyo *et al.,* 2019). Pouring the rising medium at 240 mL per tray, adding 120 ml of conidial suspension with a density of 10^6 conidia ml^{-1} prepared in distilled water continued per polybag. Disease incidence observation periodically from seven to 21 days after inoculation progressed until a significant difference in susceptibility to *F. oxysporum* attack manifested among seedlings.

Crossing of shallot Fusarium wilt-resistant and susceptible plants

Crossings between shallot Fusarium wilt-resistant and susceptible cultivars started by growing the resistant and vulnerable parents obtained in the previous experiment. The Vernalization treatment applied to the bulbs according to the method of Song *et al.* (2012) induced flowering. Treatments consisted of selfing the shallot-resistant cultivar (A), selfing the shallot-susceptible cultivar (B), and crossing the shallot-resistant and shallot-susceptible cultivars (A × B) (Figure 1).

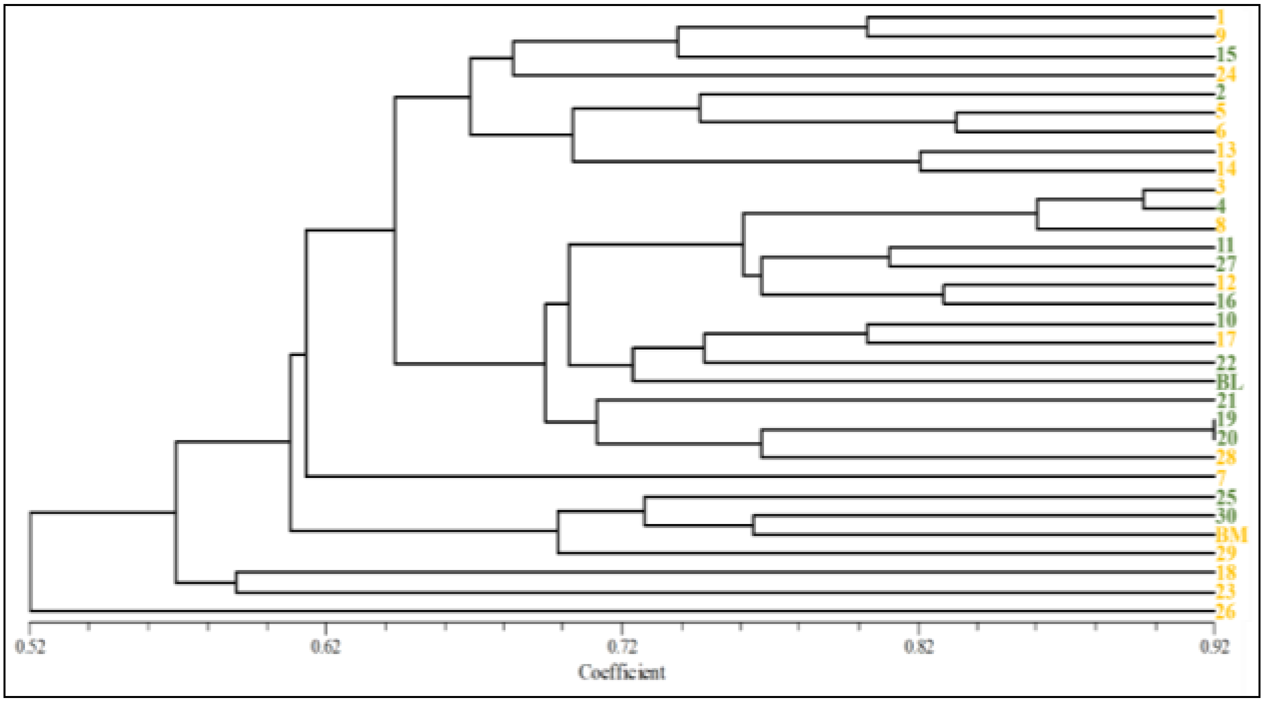

Figure 1 Dendrogram of 30 F1 populations and parental genotypes of shallot based on ISSR markers. The green-colored code represents shallot-resistant individuals, while the yellow-colored code represents shallot-susceptible individuals.

Crossings materialized 60–70 days after planting. The process of shallot crossing had pollinator insects assisting, such as fruit flies. Administering fresh shrimp fluid or a sugar solution once a week can become insect attractants (Palupi *et al.,* 2015). Variables measured included number of capsules per flower, TSS weight per plant, 100-seed weight, and germination percentage. Data analysis used an F-test at a significance level of 1%; when treatment effects appeared significant, means separation engaged Duncan's multiple range test (DMRT) at a significance level of 1%, with the selfing parent as a control.

Crossbreed resistance to Fusarium wilt

Thirty crossbred TSS seeds' planting proceeded for Fusarium wilt-resistance screening 30–40 days before seedling transplanting to a test container. When they were 40 days old, their transfer to a test container included giving an *F. oxysporum* suspension using the drenching method. Subsequently, observations were periodical until significant differences were visible between Fusarium wilt-resistant and susceptible individuals.

Analysis of crossbreeding results by ISSR

Thirty leaf samples (0.1–0.5 g each) from seedlings grown for 30–40 days from TSS became specimens for DNA isolation using the cetyltrimethylammonium bromide method (Doyle and Doyle, 1987) with modifications, according to the molecular laboratory procedures of the Center for Tropical Horticulture Studies (PKHT) IPB, Bogor, Indonesia. The isolated shallot DNA samples (5 mL) gained testing for quality by electrophoresis at 50 volts for 15 min. Observing DNA bands used a UV transilluminator.

DNA amplification employed an Applied Biosystems 2720 thermal cycler with inter-simple sequence repeat (ISSR) primers obtained from the Center for Tropical Horticulture Studies (PKHT) IPB collections (Table 1). The PCR reaction mixture consisted of 1 µl 5x DNA, 1 µl 5x primer, 6 µl 2x My Taq HS Red Mix, and 5 µl ion-free water (ddH2O). The PCR process included pre-denaturation (94 °C for 4 min), denaturation (94 °C for 30 sec), annealing (51 °C–54 °C for 45 sec), extension (72 °C for 1 min), final extension (72 °C for 5 min), and cooling (4 °C in ∞). Amplified DNA's electrophoresis was on a 1% agarose gel at 50 volts for 55 min, visualized using a UV transilluminator, and documented using a digital camera. Genetic analysis results based on molecular markers resulted in electrophoresis gels showing the presence or absence of bands, converting them into binary data, with 0 representing genotypes with no band and 1 representing genotypes with a band. The binary data processing used the NTSYS 2.0 program to determine the diversity of crossbreeding individuals resistant and susceptible (Bhardwaj *et al*., 2010).

RESULTS

Resistance of shallot to Fusarium wilt

As shown in Table 2, the six cultivars varied in their response to disease incidence after inoculation of Fusarium. Thus, for example, seven days after inoculation (DAI), 'Batu Ijo' did not exhibit any symptoms of Fusarium wilt. Furthermore, at 14 DAI, all six shallot cultivars showed symptoms and signs of fusarium

Table 1. List of ISSR primers used in this study.

No.	Primer	Base Seq.	Annealing Temp. (°C)	S. No.	Primer	Base Seq.	Annealing Temp. (°C)
1	PKBT 1	(AC)8TG	54	5	PKBT 9	(GA)9T	52
2	PKBT 3	(AC)8T	53	6	ISSRED 7	(GTC)6	51
3	PKBT 4	(AG)8AA	53	7	ISSRED 9	(CTC)5GC	52
4	PKBT 8	(GA)9C	54	8	ISSRED 25	(CCA)6	51

Table 2. Disease incidence and percentage of bulbs with Fusarium wilt disease in shallot genotypes.

Genotypes	Disease incidence (%)			Diseased bulbs (%)
	7 DAI	14 DAI	21 DAI	
Batu Ijo	0.0 b	5.6 c	19.4 c	29.5
Katumi	11.1 b	16.7 c	22.2 c	32.1
Biru Lancor	13.9 b	33.3 b	55.6 b	33.3
Kuning	13.9 b	41.7 b	69.4 b	38.5
Bima Brebes	19.4 b	72.2 a	86.1 a	41.6
Maja Cipanas	25.0 a	55.6 a	69.4 b	38.1
F - Values	3.91*	10.76**	7.42**	0.07^{tn}

Note: DAI = Days after inoculation; **. * Significant at 1% and 5% levels of probability; NS = Nonsignificant; values followed by the same lowercase letter are not significantly different based on the DMRT test at the 1% level of probability.

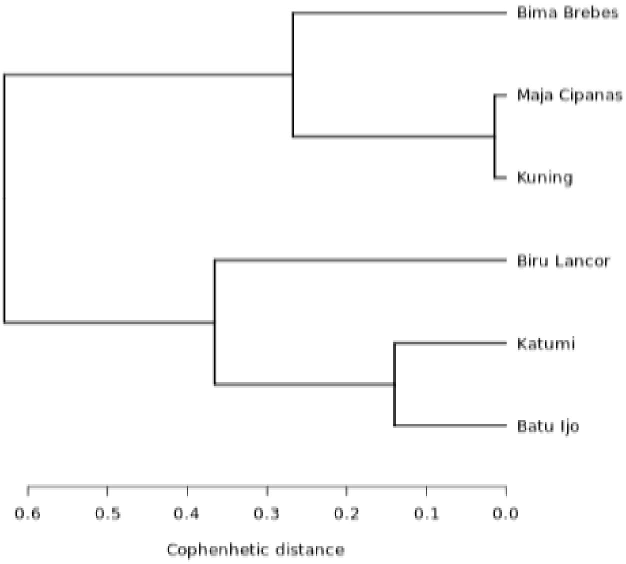

Figure 2. Dendrogram clustering of six shallot cultivars based on resistance to inoculated *F. oxysporum*.

disease (Table 2). The highest disease-incidence rate (72.2%) two weeks after inoculation (WAI) appeared in ‘Bima Brebes.’ Furthermore, at three WAI, during the last week of observation, the most affected cultivars were ‘Bima Brebes’ (86.1%), ‘Maja Cipanas’ and ‘Kuning’ (69.4%), and ‘Biru Lancor’ (55.6%). Meanwhile, the least affected cultivars were ‘Katumi’ (22.2%) and ‘Batu Ijo’ (19.4%). Fusarium wilt disease caused shallot bulbs to shrink by 29.5%–41.6% (Table 2).

Based on the disease incidence of Fusarium wilt and bulb rot after infection, the six shallot cultivars division into two clusters are as follows: group I consisted of ‘Batu Ijo,’ ‘Katumi,’ and ‘Biru Lancor,’ and group II consisted of ‘Kuning,’ ‘Maja Cipanas,’ and ‘Bima Brebes’ (Figure 2). By adjusting for disease incidence, Group I comprised cultivars with low disease incidence, and Group II included cultivars with a high disease incidence for Fusarium wilt. Cultivars ‘Batu Ijo’ and ‘Katumi’ were the most resistant to Fusarium wilt, whereas ‘Bima Brebes’ was the most susceptible. Cluster analysis revealed its usefulness for grouping shallot cultivars based on similar traits that play a vital role in breeding programs, such as selecting potential parents of shallot cultivars resistant or susceptible to Fusarium wilt.

Table 3. F-value test and means of TSS results of the two shallot populations compared with their parental genotypes.

Parameters	F – Values		Means			
	BL × BM	BM × BL	BL	BM	BL × BM	BM × BL
Number of mature capsules	22.69**	21.98**	13.2 a	10.5 b	11.0 ab	5.7 c
TSS weight/plant (g)	17.92**	0.20^{NS}	0.119 a	0.051 b	0.058 bc	0.052 bc
Weight of 100 TSS (g)	2.78^{NS}	2.71^{NS}	0.28 a	0.25 b	0.27 ab	0.22 ab
Germination (%)	136.3**	47.27**	75.00 a	56.25 b	27.08 c	18.75 c

BL, Biru Lancor; BM, Bima Brebes; **. * Significant at 1% and 5% levels of probability; NS = Nonsignificant; values followed by the same lowercase letter are not significantly different based on the DMRT test at the 1% level of probability.

Crossbreeding of shallot cultivars resistant or susceptible to Fusarium wilt

As a previous experiment showed that 'Batu Ijo' and 'Katumi' cannot bloom, cultivars 'Bima Brebes' and 'Biru Lancor' became parents of the crossbreeding instead. The mean pollen viability of 'Biru Lancor' and 'Bima Brebes' is 84.25% and 82%, respectively. Table 3 shows the results of variance analysis and the average seed yield of each parent upon selfing, 'Biru Lancor' (BL), 'Bima Brebes' (BM), and crossings ♀Biru Lancor × ♂Bima Brebes (BL × BM) and ♀Bima Brebes × ♂Biru Lancor (BM × BL), which showed lower yields compared with the selfing parents.

In crossing BL × BM, seeds harvested from the female parent, 'Biru Lancor' or 'Bima Brebes,' had a significant F value for the number of mature capsules per plant compared with the selfing parents. Furthermore, in BL × BM, the average number of mature capsules was 11 per plant, which was lower than that of the 'Biru Lancor' parent. In turn, in the BM × BL crossing, the average number of mature capsules was 5.7, which was lower than the average for both selfing parents. Seeds harvested from the female parent, 'Biru Lancor,' in the BL × BM crossing, produced a TSS weight per plant of 0.058 g, whereas the TSS weight per plant harvested from female 'Bima Brebes' in the BM × BL crossing was 0.052 g. The TSS weight of each harvested female did not significantly differ from the TSS weight per plant during the selfing of 'Bima Brebes' (0.051 g).

The crossbreeds BM × BL and BL × BM had lower TSS weights per plot than parent 'Biru Lancor' (0.119 g). As for 100-seed weight, crossbreeds BM × BL and BL × BM showed no significant differences when compared with either parent ('Biru Lancor' or 'Bima Brebes'), with an average of 0.22–0.28 g per plot. Meanwhile, on germination capacity, both crossbreeds exhibited a lower average germination rate than their parents. In both crossbreeding, BL × BM and BM × BL, the resulting germination rates were 27.08% and 18.75%, respectively, which were lower than those of the selfing parents, 'Biru Lancor' (75%) and 'Bima Brebes' (56.25%).

Analysis of resistance to Fusarium wilt in crossbreeding

Resistance analysis revealed that, of the 30 tested individuals, 17 (56.7%) resulting from the cross were susceptible to Fusarium wilt. By the end of the observation period, the surviving individuals after inoculation included numbers two, four, 10, 11, 15, 16, 19, 20, 21, 22, 25, 27, and 30, which bore resistant classification. Individuals unable to survive at the end of the observation period included numbers one, three, five, six, seven, eight, nine, 12, 13, 14, 17, 18, 23, 24, 26, 28, and 29. The symptoms and signs of attack exhibited by the individuals were relatively mild. Upon infection development in the nursery, they changed color to pale yellow, and by the end of the observation period, at 8 WAI, these plants did not survive a long time.

Seedling inoculation with *F. oxysporum* caused no severe symptoms during the first WAI because *F. oxysporum* requires a penetration time of 6–8 DAI to attack plant tissues, leading to seedling death (Widyastuti *et al.,* 2013). One WAI, some plants began to

Figure 3. Analysis of resistance of the shallot offspring from the crossing of cultivars Bima Brebes × Biru Lancor to *F. oxysporum* wilt disease. (a) TSS seedlings at 40 days after planting; (b) Plants were inoculated with *F. oxysporum* using the drenching method; (c) Seedlings showing symptoms of Fusarium wilt.

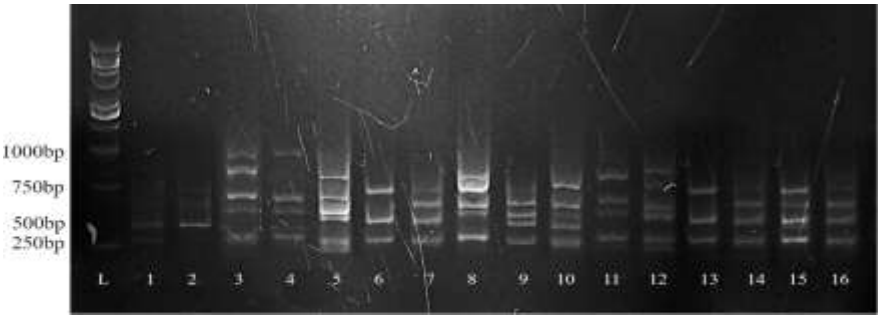

Figure 4. DNA banding patterns resulting from amplification of ISSR primer PKBT1. Patterns correspond to 16 of the 30 sampled shallot-seedlings used for the test.

show mild symptoms of Fusarium wilt, such as a change in leaf color to pale yellow (Figure 3c). Subsequently, plants' periodic observation up to eight weeks revealed 13 shallot offspring remaining alive, with 17 affected by Fusarium wilt and gradually dying.

Genetic variability analysis of crossbreds using ISSR

Molecular analysis used eight ISSR primers, and most markers produced polymorphic bands. Overall, primers amplified polymorphic bands at 94.6%, whereas monomorphic bands' amplification was only 5.4% with the number of bands for each primer (Table 4). In particular, primer PKBT 1 amplified DNA bands of 250, 500, 750, and 1000 bp (Figure 4); primer PKBT 3 amplified DNA bands of 500, 750, and 1000 bp; primer PKBT 4 amplified DNA bands of 500, 750, and 1000 bp; primer PKBT 8 amplified DNA bands of 750 and 1000 bp; primer PKBT 9 amplified DNA bands of 500, 750, and 1000 bp; primer ISSRED 7 amplified DNA bands of 500, 750, and 1000 bp; primer ISSRED 9 amplified DNA bands of 750, 1000, and 1500 bp; finally, primer ISSRED 25 amplified DNA bands of 500, 750, and 1000 bp. In addition, PKBT 1 had PIC values of 0.47, PKBT 3 (0.46), PKBT 4 (0.44), PKBT 8 (0.48), PKBT 9 (0.48), ISSRED 7 (0.39), ISSRED 9 (0.5), and ISSRED 25 (0.49) (Table 4).

Table 4. Recapitulation of amplified DNA bands and PIC values of primer 8 ISSR.

No	Primer	Number of band(s)	Polymorphic loci	Monomorphic loci	Size of amplified DNA band	PIC
1	PKBT 1	6	6	0	250-1000bp	0.47
2	PKBT 3	4	4	0	500-1000bp	0.46
3	PKBT 4	4	4	0	500-1000bp	0.44
4	PKBT 8	4	3	1	750-1000bp	0.48
5	PKBT 9	4	4	0	500-1000bp	0.48
6	ISSRED 7	3	2	1	500-1000bp	0.39
7	ISSRED 9	6	6	0	750-1500bp	0.50
8	ISSRED 25	6	6	0	500-1000bp	0.49
Total		37	94.6 %	5.4 %		----

Note: PIC= Polymorphism Information Content.

The analysis of diversity based on ISSR markers revealed three major groups, where group II showed the lowest percentage of Fusarium wilt-affected plants, i.e., 35.7%, and groups I and II showed symptoms of Fusarium wilt in 77.8% and 71.4% of the population, respectively. Within group II, green-colored individuals indicated resistance to Fusarium wilt (Figure 3). The resistant parent 'Biru Lancor' was also in Group II. These results expect that the male parent's resistance to Fusarium wilt could transmit disease resistance to the offspring.

DISCUSSION

Analysis of the resistance of shallot cultivars to Fusarium wilt showed that 'Bima Brebes' suffered the highest disease incidence rate upon inoculation, whereas 'Batu Ijo' showed the lowest. These results agree with previous research conducted by Supyani *et al.* (2021), who recorded 'Bima Brebes' showing the highest incidence of Fusarium wilt disease during the rainy season among the shallot cultivars tested in their study. Moreover, based on further studies over three consecutive years, the cultivar 'Bima Brebes' had an increasing disease incidence (32.14%). Meanwhile, Aprilia *et al.* (2020) and Maharijaya *et al*. (2023) also reported that the 'Batu Ijo' cultivar was Fusarium wilt-disease resistant.

The results described herein revealed that the symptoms of Fusarium wilt observed in the field after inoculation with the *F. oxysporum* isolate were leaf wilting and yellowing, followed by bulb rot. According to Herlina *et al.* (2021), the symptoms of Fusarium wilt in Indonesia differ slightly from those in other countries. Specifically, in Indonesia, the disease begins with leaf necrosis (twisting), after which plants wilt and rot, resulting in no bulb formation. Moreover, the plant's infection affected bulb development because the disease starting to affect the plant disturbs all organs, from the leaves to the roots (Le *et al.,* 2021). Thus, the leaves began to turn yellow and curl, followed by the rotting of the shallot bulb. The severity of bulb rot increases as fungal mycelia develops in the soil. Therefore, to reduce the loss of shallot bulbs owing to disease severity, several preventive measures can start, such as careful planning of planting based on the knowledge related to the evaluation of the planting season and the use of a combination of fungicides as control agents (Marlin *et al*., 2018; Degani *et al.,* 2022).

In this study, crossbreeding between shallot cultivars 'Biru Lancor' and 'Bima Brebes' proceeded successfully, suggesting that the parents had no barrier that might interfere with hybridization. Compatibility refers to the ability of a plant to produce seeds through self-pollination or cross-pollination with the help of nature (insects) or humans (Quinaluisa *et al.,* 2021). Specifically, seed yields of the BL × BM and BM × BL crosses were lower than those of the selfing of both parents. A higher yield after selfing than after crossbreeding is often clear evidence of self-compatibility. According to Cropano *et al.* (2021), self-compatibility is a genetic or environmental mechanism whereby a plant can fertilize its ovules with its pollen without the intervention of pollen from another plant.

Plants with self-compatibility are often helpful in forming primary populations of hybrid progenies. Previous studies have suggested that selecting self-compatible individuals or clones of cocoa is the best strategy for their maintenance and evaluation in subsequent years (Quinaluisa *et al*., 2021). In addition, to improve buckwheat plant yield, wide hybridization has continued by utilizing other individuals from the same family with high self-compatibility rates (Woo *et al.,* 2018). Furthermore, a low seed germination rate resulted in the fact that the seeds produced from a cross have a higher chance of losing their viability (Baskin and Baskin, 2015). In this study, low seed yield may have been due to the sensitivity of shallot flowering to low temperatures and the resulting physiological conditions of the plants (Krontal *et al.,* 2000; Billa *et al*., 2022; Fairuzia *et al*., 2022).

The third experiment tested the resistance of the 'Bima Brebes' × 'Biru Lancor' cross to *F. oxysporum*. In this test, 17 out of 30 individuals showed symptoms of Fusarium wilt. The symptoms and signs of attack exhibited by these plants were still considerably mild because the plants attacked in the nursery changed color to pale yellow, and they died soon after the end of the observation period. These results are consistent with the findings of Southwood *et al.* (2015), who reported that shallots sustain effects from Fusarium at low frequency at the nursery stage, and disease symptoms were not visible then. However, the severity of Fusarium wilt increased from the sixth week, causing susceptible plants to die.

Genetic variability analysis using ISSR primers yielded polymorphism information content (PIC) values that helped assess the strength of each primer in the tested individuals (Manzila *et al.,* 2021). Among eight primers tested, ISSRED 9 had the highest PIC value. PIC values, ranging from 0.25 to 0.5, are often informative for primer strength (Dalimunthe *et al.,* 2020). Clustering analysis based on ISSR markers and results of the Fusarium wilt resistance test showed that group II, which consisted of plants from the cross-population 'Bima Brebes' × 'Biru Lancor,' showed similarities to the parent cultivar 'Biru Lancor' (resistant to Fusarium wilt) and experienced a lower level of infection than in the other two groups. Confirmation of hybrid authenticity through ISSR reportedly indicates that progenies that inherit more fragments from the male parent will be the same genotype as the male parent in the analysis (Bianco *et al.,* 2011).

In this study, some F_1 seeds attained production by successful crossing between two different parents. Cultivar 'Biru Lancor,' the male parent in the cross, produced most of the population resistant to Fusarium wilt. These findings suggest that, when forming a new population of Fusarium wilt-resistant shallots, using a male parent with disease resistance is most necessary, as long as there is no maternal-effect interaction from both parental crosses. In agreement, Santamaria *et al.* (2018) reported that total plant resistance may be due to a single dominant gene. Partial resistance of a plant, which has both vulnerable and resistant roles, requires conducting test crosses to determine the dominant genes' roles involved. The incidence rate and severity of Fusarium wilt disease depend on environmental conditions and planting season. A resistant population has an additive inheritance of basal Fusarium rot-resistance genes (Mandal and Cramer, 2021). Short-lived plant resistance has recessive genes typically under control (Ssali *et al.,* 2013). Partial plant resistance needs to develop through multilocation and multi-season testing to validate the development of cultivar resistance. Advanced molecular analyses can also help elucidate various mechanisms involved in plant disease resistance. Thus, specific information regarding the roles of genes involved in plant resistance to particular diseases is obtainable.

CONCLUSIONS

Analysis of plant resistance against Fusarium wilt showed that shallot cultivars 'Batu Ijo' and 'Katumi' were the most resistant cultivars, whereas cultivar 'Bima Brebes' was susceptible. Crossbreeding between moderately resistant cultivars 'Biru Lancor' and 'Bima Brebes' resulted in a lower capsule yield, lower TSS weight per plant, and lower germination rate than the selfing of either parent owing to the self-compatibility of both cultivars. Molecular analysis using ISSR successfully divided the shallot crossbreeding populations into three main groups, of which the second group had the lowest Fusarium wilt incidence rate (35.7 %). This finding indicates that crossbreeding between the selected resistant and susceptible cultivars successfully formed a population that was relatively resistant to Fusarium wilt compared with the male parent.

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