Caraka Tani: Journal of Sustainable Agriculture
ISSN 2613-9456 (Print) 2599-2570 (Online)

Caraka Tani: Journal of Sustainable Agriculture, 37(2), 333-343, 2022
URL: https://jurnal.uns.ac.id/carakatani/article/view/56341
DOI: http://dx.doi.org/10.20961/carakatani.v37i2.56341

Performance Evaluation of Induced Mutagenesis using Colchicine and EMS Solution on Cowpea M3 Purple and Mung Bean Vima1 to Increase Resistance

I Gede Ketut Susrama*, Ni Made Trigunasih, I Ketut Suada and Ni Nyoman Ari Mayadewi

Department of Agroecotechnology, Faculty of Agriculture, Universitas Udayana, Denpasar, Indonesia

**Corresponding author:* ketutsusrama@unud.ac.id

Abstract

Induced mutagenesis is one way to improve the quality of crops, especially to increase the resistance to pests and diseases. This research aimed to determine colchicine and ethyl methane sulfonate (EMS) mutagenesis on cowpea M3 purple and mung bean Vima1, in concatenation for developing resistant crops. This research consists of four packages: (1) first package (cowpea M3 purple seeds treated with colchicine solution, (2) second package (cowpea M3 purple seeds treated with EMS solution), (3) third package (mung bean Vima1 seeds treated with colchicine solution) and (4) fourth package (mung bean Vima1 seeds treated with EMS solution). The results of this research revealed that induced mutagenesis by colchicine solution treatment reduced the incidence of bean leaf beetles up to 19% on cowpea M3 purple, as well leaf miners by 5% to 9% and bean leaf beetles up to 5% on mung bean Vima1. Treatment of EMS solution decreased the incidence of bean leaf beetles by 17% on cowpea M3 purple and pink mealybug by 5% to 15% on mung bean Vima1. Induced mutagenesis using EMS solution significantly decreased vegetable leaf miner incidence by 33% to 93% or 71% on average. Colchicine and EMS solution treatment caused aphid attacks in cowpea M3 purple, particularly in pods. The attacks did not happen on leaves and aphid incidence in pods was slower than in control. Some promising mutant candidates were found from this research that will be used in further crop development studies. It is expected that the method and results of this research could inspire faster development of resistant crops.

Keywords: chemical mutagens; crop improvement; ethyl methane sulfonate (EMS); mutation breeding; pest and disease resistance

Cite this as: Susrama, I. G. K., Trigunasih, N. M., Suada, I. K., & Mayadewi, N. N. A. (2022). Performance Evaluation of Induced Mutagenesis using Colchicine and EMS Solution on Cowpea M3 Purple and Mung Bean Vima1 to Increase Resistance. *Caraka Tani: Journal of Sustainable Agriculture*, *37*(2), 333-343. doi: http://dx.doi.org/10.20961/carakatani.v37i2.56341

INTRODUCTION

Various ways can be carried out to improve the quality of crops, especially in developing resistant plants, one of which is by inducing mutagenesis. Mutagenesis induction is a very potential way in efforts to improve plant quality and it is a promising approach to creating plant genetic variability (Roychowdhury and Tah, 2011; Oladosu et al., 2015; Melsen et al., 2021). Mutagenesis induction has been done for decades in obtaining crops with higher resistance to pests and diseases, and finding new and distinctive crop quality characteristics that have higher academic and economic values. Various success has been achieved by many researchers who have successfully disseminated new and better crop varieties through induced mutagenesis.

* *Received for publication November 8, 2021*
Accepted after corrections July 18, 2022

In addition, crop improvement, especially developing resistant plants through induced mutagenesis, is interesting and promising.

Developing resistant plants is an important way of reducing the use of pesticides although pesticides are important components of integrated pest management and without pesticide use, it would be a production loss of fruit, vegetable and cereal of approximately 78%, 54% and 32%, respectively (Tudi et al., 2021). However, pesticides are poisons, which are dangerous to abiotic and biotic environments (Dennis et al., 2018). Therefore, pesticide use must be reduced for agriculture sustainability. It is urgent to minimize crop yield losses resulting from pest incidences and decrease the impacts of pest management activities on human health and the environment through a more resilient and sustainable approach (Baker et al., 2020). In reducing pesticide uses, it is necessary to provide other control options that are effective as substitutes. Planting resistant crop as an environmentally friendly component of integrated pest and disease management is a sustainable agricultural practice. Crop pests and diseases management by planting resistant crops are a wise and foremost choice in plant cultivation, considering that planting resistant plants will eliminate pest and disease attacks and then if there are no pest and disease incidences, farmers automatically do not need to use pesticide. Given the importance of planting resistant crops in efforts to manage crop pests and diseases, such as in managing green stink bug, *Acrosternum hilare* (Kamminga et al., 2012), development of resistant crops is necessary to continue. In addition, better understanding of plant defense mechanism, pests and pathogens adaptability to its host plant, and plant genetic modification for developing plant resistance are greatly importance for sustainable agriculture.

In this study, the authors carried out induced mutagenesis research on cowpea and mung beans. Cowpea could be a solution to solve the problem of dry land, dry climate areas and or drought-prone areas, and it is useful for increasing soil fertility (Alemu et al., 2016; Salama et al., 2022). Beside cowpea, mung bean cultivation is another choice for facing drought (Daryanto et al., 2015), as mentioned that legumes, such as green gram (*Vigna radiata*) and cowpea (*Vigna unguiculata*), were found to experience higher drought-induced yield reduction compared to lentil (*Lens culinaris*), groundnut (*Arachis hypogaea*) and pigeon pea (*Cajanus cajan*). Mung bean, like soybean and peanut, is a tropical plant that is in high demand by the public for a variety of reasons, particularly for food and health supplements. Mung bean sprouts contain anti-aging active ingredients (Kaura and Parle, 2017), it was reported that mung bean contains antioxidant active ingredients during the germination process (Xue et al., 2016).

Colchicine and ethyl methane sulfonate (EMS) were the two mutagens used in this study. Colchicine has been widely used to develop tetraploid and mixoploid plants (Atichart, 2013; de Carvalho et al., 2016; Mori et al., 2016; Julião et al., 2020) and to increase plant genetic variation (El-Nashar and Ammar, 2016). EMS is also frequently used as a chemical mutagen for plant mutagenesis considering it causes a high frequency of nucleotide substitution (Talebi et al., 2012). Al-Kubati et al. (2021) succeeds in developing resistant bottle gourd bitter melon to zucchini yellow mosaic virus disease through EMS mutagenesis. Further, Khursheed et al. (2018) reported that, in faba bean (*Vicia faba* L.), EMS was more effective than gamma rays in inducing mutagenesis.

Hydrogen peroxide (H_2O_2) solution used in this induced mutagenesis research as a solvent. H_2O_2 is an important signaling molecule in plant developmental processes and stress responses (Niu and Liao, 2016; Tian et al., 2018) and oxidizes cysteine to sulfenic acid. The modification initiates a thiol-based redox reaction and modifies target enzymes, kinase receptor and transcription factors (Smirnoff and Arnaud, 2019). In cowpea, treatment of H_2O_2 did not cause pod color change and treatment of colchicine in water as its solvent did not cause pod color change, as well. However, treatment of colchicine in H_2O_2 caused pod color change from green to purple (Susrama and Wirawan, 2017). The results suggested that H_2O_2 is an important substance in plant induced mutagenesis. Furthermore, colchicine and EMS mutagenesis in H_2O_2 solution as a solvent are innovations in the process of mutagenesis induction especially for resistant plant development. The purpose of this study is to determine the induced mutagenesis effects of colchicine and EMS treatment in H_2O_2 as its solvent on cowpea M3 purple and mung bean Vima1 for developing resistant crop.

MATERIALS AND METHOD

Research location

This research was carried out from May to August 2020 in Taman Harum Sub-village, Padang Sambian Village, Denpasar Barat Sub-district, Denpasar, Bali, Indonesia. The experimental site is located at 8°39’05.1” S and 115°10’55.1” E and approximately 35 m above sea level.

Research materials and equipment

Materials used in this research were cowpea M3 purple seeds of authors previous research (Susrama and Pradnyawathi, 2020), mung bean Vima1, colchicine, EMS and H_2O_2. Equipment used includes measuring cylinder, beaker glass, electronic scale, petri dish, spatula, pipettes and cultivation tools.

Procedures

Colchicine and EMS were used for inducing mutagenesis in cowpea M3 purple and mung bean Vima1. This research was divided into four packages. Each package of this research was arranged in randomized complete block design consisting of two treatments and a control, with replicates between 12 to 22 times. The first package consisted of a colchicine in 0.1% H_2O_2 treatment on cowpea M3 purple seeds with doses of: (1) 25 seeds soaked in 0.001% colchicine solution, (2) 25 seeds soaked in 0.01% colchicine solution, (3) 25 seeds soaked in 0.1% H_2O_2 as control. The second package was EMS in 0.1% H_2O_2 treatment on cowpea M3 purple seeds with doses of: (1) 25 seeds soaked in 0.01% EMS solution, (2) 25 seeds soaked in 0.1% EMS solution, (3) 25 seeds soaked in 0.1% H_2O_2 as control. The third package was treatment on mung bean Vima1 seeds with colchicine in 0.1% H_2O_2 using the doses of: (1) 25 seeds soaked in 0.0001% colchicine solution, (2) 25 seeds soaked in 0.001% colchicine solution, (3) 25 seeds soaked in 0.1% H_2O_2 as control. The fourth package was treatment on mung bean Vima1 seeds with EMS in 0.1% H_2O_2 using the doses of: (1) 25 seeds soaked in 0.0001% EMS solution, (2) 25 seeds soaked in 0.001% EMS solution, (3) 25 seeds soaked in 0.1% H_2O_2 as control. Each treatment was carried out by immersion in a petri dish for 24 hours. All seeds were then planted on a land that had been prepared, separately according to the package. Crops that grew well were observed as samples.

The observed parameters were crop morphological changes and time series data of pests and diseases incidences starting from the time of germination to harvesting time. Any unusual morphological changes, especially beneficial morphological changes, were recorded. Pests and diseases that attack all plant samples were observed and identified based on the visual symptoms *in situ*, without any artificial inoculation.

Statistical analysis

Prior to statistical analysis, all data were transformed with square root +0.5 for normalizing data statistically. Then, data were statistically analyzed through one-way analysis of variance (ANOVA) and Tukey’s honest significant difference test by IBM SPSS 25.

RESULTS AND DISCUSSION

Colchicine in H_2O_2 treatment on cowpea M3 purple

Incidence of bean leaf beetles (Cerotoma trifurcata)

Symptoms of leaf damage in the form of neat hollows in attacked leaves (Figure 1) were found in the colchicine solution treated cowpea M3 purple and in control as well. The symptoms were diagnosed due to insect pest attack of bean leaf beetles (*C. trifurcata*). When 0.01% colchicine solution treated cowpea M3 purple was compared to the control group, the incidence of bean leaf beetles was reduced by up to 19%, or 12.7% on average (Table 1). Colchicine treatments cause an increase in the number of chromosomes in many kinds of plants and affect the traits, such as on cotton (Mustafa et al., 2017), golden berry (Comlekcioglu and Ozden, 2019) and pepper (Tammu et al., 2021). The increase in number of chromosomes causes plants to have advantages of polyploid plants, namely the emergence of heterosis characteristics with greater resistance to biotic and abiotic stresses (Tsukaya, 2013) and increases cell size, organ size and at the same time increase the plant’s tolerance to environmental stresses (Wang et al., 2017).

Figure 1. Symptoms of bean leaf beetles

Incidence of cowpea aphid (Aphis craccivora) on cowpea pods

The authors previous study, it was observed that cowpea aphid incidence nearly always occurred in the vegetative stage of cowpea M1 purple and cowpea M2 purple specifically attacking shoots and leaves. Cowpea aphid attacks commonly started in the early weeks on shoots or leaves at week III or IV. In this research, cowpea aphids were not found in the vegetative stage of cowpea M3 purple. Cowpea aphid attacks on pods occurred at 66 days after planting. The incidence of cowpea aphid on cowpea pods was seven days slower than the incidence in the control and its attacks percentage on pods was just up to 5% or in average 3.3% reduced (Table 2). Although this result is statistically unsignificant, colchicine mutagenesis increased the resistance of cowpea M3 purple in the absence of aphids in the vegetative growth phase. This increase in resistance was probably due to the increase of pod's flavonoid content, which produced the purple color of the pods. Kasmiyati et al. (2020) reported induced polyploidy using colchicine be able to increase flavonoid content. Furthermore, flavonoid affect the behavior of aphid especially it could decrease sap ingestion activity (Goławska et al., 2014; Stec et al., 2021). Considering that aphid is a kind of pest which is very often attacking beans and very difficult to manage, this result is an important progress in developing an aphid-resistant crop.

EMS in H_2O_2 treatment on cowpea M3 purple

Incidence of vegetable leaf miner (Liriomyza spp.)

The authors found the symptoms of vegetable leaf miner (*Liriomyza spp.*), which appeared seven days after planting. Cowpea from seeds treated with 0.1% EMS solution showed no vegetable leaf miner symptoms, while in control cowpea, there was 33% attack percentage at 49 days after planting, 87% at 56 days after planting and 93% at 63 days after planting. Induced mutagenesis with 0.1% EMS solution significantly decreased vegetable leaf miner incidence, ranging from 33% to 93% or in average 71% (Table 3). This change in resistance is likely

Table 1. Incidence of bean leaf beetles on cowpea M3 purple

Days after planting	Number of cowpeas observed	Control	Attack percentage		Incidence reduction (%)	
			Colchicine 0.001%	Colchicine 0.01%	Colchicine 0.001%	Colchicine 0.01%
35	12	8	18	8	-10	0
42	12	27	27	8	0	19
49	12	27	27	8	0	19
	Average^{ns}	20.7	24	8	-3.3	12.7

Note: ns = not significant at 5% probability level ($p < 0.005$)

Table 2. Incidence of aphids on cowpea M3 purple pod

Days after planting	Number of cowpea pods observed	Control	Attack percentage*)		Incidence reduction (%)	
			Colchicine 0.001%	Colchicine 0.01%	Colchicine 0.001%	Colchicine 0.01%
56	20	0	0	0	0	0
63	20	5	0	0	5	0
70	20	10	5	5	5	5
	Average^{ns}	5.0	1.7	1.7	3.3	3.3

Note: ns = not significant at 5% probability level ($p < 0.005$); * = percentage of cowpea pods attacked by aphids from observed pods

Table 3. Incidence of vegetable leaf miners (*Liriomyza spp.*) on cowpea M3 purple

Days after planting	Number of cowpea pods observed	Control	Attack percentage		Incidence reduction (%)	
			EMS 0.01%	EMS 0.1%	EMS 0.01%	EMS 0.1%
49	15	33	27	0	6	33
56	15	87	27	0	60	87
63	15	93	47	0	46	93
	Average*	71^{a}	33.7^{b}	0^{b}	37.3	71

Note: * = significant at 5% probability level (p < 0.005). Numbers in one column followed by the same letter are not significantly different based on Tukey's test result

Table 4. Incidence of bean leaf beetle on cowpea M3 purple

Days after planting	Number of cowpeas observed	Control	Attack percentage		Incidence reduction (%)	
			EMS 0.01%	EMS 0.1%	EMS 0.01%	EMS 0.1%
28	12	0	0	0	0	0
35	12	8	16	8	-8	0
42	12	25	25	8	0	17
49	12	25	25	8	0	17
	Average^{ns}	14.5	16.5	6.0	-2.0	8.5

Note: ns = not significant at 5% probability level (p < 0.005)

due to the influence of EMS mutagenesis on the leaf anatomical structure, as reported by Arisha et al. (2015), that EMS treatment increased xylem tissue, spongy tissue and palisade thickness in leaf blade and increased collenchyma in leaf's midrib. Plant resistance changes may also be attributed to an increase in the content of acyl sugar, a secondary plant secondary metabolite associated with innate plant resistance against herbivory, based on the research result of Dias et al. (2013) that plant containing higher acyl sugars was having higher resistance to leaf miner. This result suggests that mutagenesis using EMS in H_2O_2 cowpea that is resistant to leaf miners.

Symptom of bean leaf beetles (C. trifurcata)

The symptoms of bean leaf beetles occurred from 35 to 49 days after planting both in EMS treated cowpea M3 purple and in control. The percentage of attack decreased up to 17% in 0.1% EMS treated cowpea M3 purple compared to control or on average 8.5% (Table 4).

Incidence of cowpea aphid (A. craccivora) on pod of cowpea M3 purple

Cowpea aphid incidence occurred after pods formation at 62 days after planting and cowpea aphid attacks did not occur on leaves. Attacks of cowpea aphids on young pods were caused the pods to shrivel, wilt and dry out. Cowpea aphid attack on pods after seed formation, on the other hand, caused no serious damage to the seeds, only blackening of the surface of pod skin at the point of stylet insertion. Nair et al. (2019) stated that polyploidy could cause changes in cell wall composition. The changes may have caused a shift in the resistance of cowpea M3 purple to cowpea aphid. Cowpea aphid preference for leaves appears to have decreased due to chemical content changes, as attacks on leaves did not occur. Cowpea aphid incidence in 0.1% EMS treated cowpea M3 purple pods occurred two weeks after control cowpea aphid incidence and the percentage of attack was 11.7% lower on average (Table 5).

Colchicine in H_2O_2 treatment on mung bean Vima1

Symptom of bean leaf beetles (C. trifurcata)

Symptom of bean leaf beetle attack on mung bean Vima1 (Figure 2) appeared from the beginning of growth, starting from the first week to the third week on both colchicine treated and control mung bean. Bean leaf beetle incidence on 0.0001% colchicine solution treated mung bean Vima1 was statistically insignificant compared to the control. However, on mung bean Vima1 treated with 0.001% colchicine, the attack percentage was reduced by 3.3% on average (Table 6). Those results suggested that higher colchicine concentrations should be applied in the future researches for achieving a better result. On the other hand, other mutagens and other solvents should be tried for mutagenesis induction in developing new resistant mung bean against bean leaf beetles.

Figure 2. Bean leaf beetle symptoms in mung bean Vima1

Incidence of leaf miners (Liriomyza spp.) on mung bean Vima1

The presence of leaf miners was evidenced in both control and colchicine-treated mung beans. The symptom of leaf miner attack on mung bean Vima1 treated with colchicine at 0.0001% was higher than that of in the control; however, in mung bean treated with 0.001% colchicine, the symptom was lower up to 9% or on average 4.8%, despite being statistically insignificant (Table 7). Succinic acid was reported to show a strong relation to leaf miner resistance (Chrigui et al., 2021) and thus, for the future induced mutagenesis research, succinic acid may be used as a mutagen or as a solvent in treating a mutagenic agent.

Mung bean yellow mosaic virus (MYMV) disease incidence in mung bean Vima1

Yellow mosaic virus disease is a kind of plant disease that often attack mung bean (Karthikeyan et al., 2014) and this disease was reported to attack mung bean all over the world (Mishra et al., 2020). The incidence of MYMV was found in colchicine treated mung beans and in control. Treatment of 0.001% colchicine during the early growth reduced incidence of MYMV in mung bean Vima1 by 9% to 22%, with an average of 9.5%. Unfortunately, all crop samples were infected 70 days after planting (Table 8).

EMS in H_2O_2 treatment on mung bean Vima1

Incidence of pink mealybug (Maconellicoccus hirsutus)

Pink mealybug (Figure 3) has easily identifiable morphological characteristics, including pink eggs, nymphs and adult stages, both winged and unwinged. Meanwhile, the majority of mealybugs found in beans are white in color. Pink mealybug infestations were discovered in both control and EMS solution treated mung beans. Treatment with 0.001% EMS solution reduced pink mealybug infestation by 5% to 15% (Table 9), which was statistically insignificant.

Table 5. Aphid incidence on pod of cowpea M3 purple

Days after planting	Number of cowpea pods observed	Control	Attack percentage		Incidence reduction (%)	
			EMS 0.01%	EMS 0.1%	EMS 0.01%	EMS 0.1%
56	20	10	5	0	5	10
63	20	15	25	0	-10	15
70	20	20	25	10	-5	10
	Average^{ns}	15.0	18.3	3.3	-3.3	11.7

Note: ns = not significant at 5% probability level ($p < 0.005$)

Table 6. Incidence of bean leaf beetles on mung bean Vima1

Days after planting	Number of mung beans observed	Control	Attack percentage		Incidence reduction (%)	
			Colchicine 0.0001%	Colchicine 0.001%	Colchicine 0.0001%	Colchicine 0.001%
7	22	9	14	9	-5	0
14	22	23	23	18	0	5
21	22	23	23	18	0	5
	Average^{ns}	18.3	20	15	-1.7	3.3

Note: ns = not significant at 5% probability level ($p < 0.005$)

Table 7. Incidence of leaf miners on mung bean Vima1

Days after planting	Number of mung beans observed	Control	Attack percentage		Incidence reduction (%)	
			Colchicine 0.0001%	Colchicine 0.001%	Colchicine 0.0001%	Colchicine 0.001%
7	22	14	27	9	-13	5
14	22	14	27	9	-13	5
21	22	41	59	32	-18	9
	Average^{ns}	17.3	28.3	12.5	-11.0	4.8

Note: ns = not significant at 5% probability level ($p < 0.005$)

Table 8. Incidence of MYMV in mung bean Vima1

Days after planting	Number of mung beans observed	Control	Attack percentage		Incidence reduction (%)	
			Colchicine 0.0001%	Colchicine 0.001%	Colchicine 0.0001%	Colchicine 0.001%
35	22	27	27	14	0	13
42	22	36	59	14	-23	22
49	22	55	68	41	-13	14
56	22	77	82	68	-5	9
63	22	86	91	77	-5	9
70	22	100	100	100	0	0
	Average^{ns}	54.4	61	44.9	-6.6	9.5

Note: ns = not significant at 5% probability level ($p < 0.005$)

Incidence of MYMV in mung bean Vima1

MYMV (Figure 4) was found in both EMS solution treated and control mung beans, with an attack percentage of 43.4% in control, 40.4% in 0.0001% EMS treatment and 38.1% in 0.001% EMS solution treatment. EMS in H_2O_2 mutagenesis reduced MYMV incidence by 3% in 0.0001% EMS treatment and 5.3% in 0.001% EMS treatment. Secondary metabolites are plant innate immunity that plants use to protect themselves from disease pathogens. These secondary metabolites were thought to be in planta antibiotics (Piasecka et al., 2015). All control mung bean Vima1 were infected 70 days after planting, whereas 10% EMS of 0.001% treated mung bean Vima1 showed no symptoms (Table 10). Uninfected mung bean Vima1 pod seeds were stored for future research.

Figure 3. Pink mealybug

Figure 4. Symptoms of MYMV

Table 9. Percentage of pink mealybug attacks on mung bean Vima1

Days after planting	Number of mung beans observed	Control	Attack percentage		Incidence reduction (%)	
			EMS 0.0001%	EMS 0.001%	EMS 0.0001%	EMS 0.001%
28	20	5	0	0	5	0
35	20	15	20	0	-5	15
42	20	25	30	20	-5	5
49	20	35	45	30	-15	5
	Average^{ns}	16.0	19.0	10.0	-3	3

Note: ns = not significant at 5% probability level ($p < 0.005$)

Table 10. Percentage of MYMV attacks in mung bean Vima1

Days after planting	Number of mung beans observed	Control	Attack percentage		Incidence reduction (%)	
			EMS 0.0001%	EMS 0.001%	EMS 0.0001%	EMS 0.001%
35	30	7	3	7	4	0
42	30	17	17	10	0	7
49	30	37	33	30	4	7
56	30	60	57	53	3	7
63	30	83	83	77	0	6
70	30	100	90	90	10	10
	Average^{ns}	43.4	40.4	38.1	3	5.3

Note: ns = not significant at 5% probability level ($p < 0.005$)

CONCLUSIONS

Colchicine-induced mutagenesis in H_2O_2 treatment reduced incidence of bean leaf beetles on cowpea M3 purple, as well as leaf miners and bean leaf beetles on mung bean Vima1. Treatment of EMS in H_2O_2 lowered the incidence of bean leaf beetles on cowpea M3 purple and pink mealybug on mung bean Vima1. Induced mutagenesis with 0.1% EMS solution significantly decreased vegetable leaf miner incidence of 33% to 93%, with 71% on average. Colchicine and EMS in H_2O_2 treatment caused aphid attack on cowpea M3 purple just occurred on pods but not on leaves and aphid incidence on pods of treated cowpea M3 purple were slower than control.

ACKNOWLEDGEMENT

This research was carried out with financial support from the Universitas Udayana Research Center for the fiscal year of 2020. On this occasion, the authors would like to express highly gratitude to all colleagues and friends, who have contributed to and helped with this research. Special thanks go to Professor I Wayan Supartha for his insightful suggestions.

REFERENCES

Alemu, M., Asfaw, Z., Woldu, Z., Fenta, B. A., & Medvecky, B. (2016). Cowpea (*Vigna unguiculata* (L.) Walp.) (Fabaceae) landrace diversity in Northern Ethiopia. *International Journal of Biodiversity and Conservation*, *8*(11), 297–309. https://doi.org/10.5897/ijbc2016.0946

Al-Kubati, A. M. S., Kang, B., Liu, L., Abbas, A., & Gu, Q. (2021). Development of bottle gourd lines resistant to zucchini yellow mosaic virus using ethyl methanesulfonate mutagenesis. *HortScience*, *56*(8), 909–914. https://doi.org/10.21273/HORTSCI15898-21

Arisha, M. H., Shah, S. N. M., Gong, Z. H., Jing, H., Li, C., & Zhang, H. X. (2015). Ethyl methane sulfonate induced mutations in M2 generation and physiological variations in M1 generation of peppers (*Capsicum annuum* L.). *Frontiers in Plant Science*, *6*(399), 1–11. https://doi.org/10.3389/fpls.2015.00399

Atichart, P. (2013). Polyploid induction by colchicine treatments and plant regeneration of *Dendrobium chrysotoxum*. *Thai Journal of Agricultural Science, 46*(1), 59–63. Retrieved from https://www.cabdirect.org/cabdirect/abstract/20133297863

Baker, B. P., Green, T. A., & Loker, A. J. (2020). Biological control and integrated pest management in organic and conventional systems. *Biological Control*, *140*, 104095. https://doi.org/10.1016/j.biocontrol.2019.104095

Chrigui, N., Sari, D., Sari, H., Eker, T., Cengiz, M. F., Ikten, C., & Toker, C. (2021). Introgression of resistance to leafminer (*Liriomyza cicerina* Rondani) from *Cicer reticulatum* Ladiz. to *C. arietinum* L. and relationships between potential biochemical selection criteria. *Agronomy*, *11*(1), 57. https://doi.org/10.3390/agronomy11010057

Comlekcioglu, N., & Ozden, M. (2019). Polyploidy induction by colchicine treatment in golden berry (*Physalis peruviana*), and effects of polyploidy on some traits. *Journal of Animal and Plant Sciences*, *29*(5), 1336–1343. Retrieved from http://www.thejaps.org.pk/docs/v-29-05/14.pdf

Daryanto, S., Wang, L., & Jacinthe, P. A. (2015). Global synthesis of drought effects on food legume production. *PLoS ONE*, *10*(6), e0127401. https://doi.org/10.1371/journal.pone.0127401

de Carvalho, M. de J. d. S., Gomes, V. B., Souza, A. da S., Aud, F. F., Santos-Serejo, J. A., & Oliveira, E. J. (2016). Inducing autotetraploids in cassava using oryzalin and colchicine and their *in vitro* morphophysiological effects. *Genetics and Molecular Research*, *15*(2), gmr.15028281. https://doi.org/10.4238/gmr.15028281

Dennis, P. G., Kukulies, T., Forstner, C., Orton, T. G., & Pattison, A. B. (2018). The effects of glyphosate, glufosinate, paraquat and paraquat-diquat on soil microbial activity and bacterial, archaeal and nematode diversity. *Scientific Reports*, *8*(1), 2119. https://doi.org/10.1038/s41598-018-20589-6

Dias, D. M., Resende, J. T. V., Faria, M. V., Camargo, L. K. P., Chagas, R. R., & Lima, I. P. (2013). Selection of processing tomato genotypes with high acyl sugar content that are resistant to the tomato pinworm. *Genetics and Molecular Research*, *12*(1), 381–389. https://doi.org/10.4238/2013.February.8.2

El-Nashar, Y. I., & Ammar, M. H. (2016). Mutagenic influences of colchicine on phenological and molecular diversity of *Calendula officinalis* L. *Genetics and Molecular Research*, *15*(2), gmr.15027745. https://doi.org/10.4238/gmr.15027745

Goławska, S., Sprawka, I., Łukasik, I., & Goławski, A. (2014). Are naringenin and quercetin useful chemicals in pest-management strategies? *Journal of Pest Science*, *87*(1), 173–180. https://doi.org/10.1007/s10340-013-0535-5

Julião, S. A., Ribeiro, C. do V., Lopes, J. M. L., Matos, E. M. de, Reis, A. C., Peixoto, P. H. P., Machado, M. A., Azevedo, A. L. S., Grazul, R. M., Campos, J. M. S. de, & Viccini, L. F. (2020). Induction of synthetic polyploids and assessment of genomic stability in *Lippia alba*. *Frontiers in Plant Science*, *11*, 292. https://doi.org/10.3389/fpls.2020.00292

Kamminga, K. L., Koppel, A. L., Jr. D. A. H., & Kuhar, T. P. (2012). Biology and management of the green stink bug. *Journal of Integrated Pest Management*, *3*(3), C1–C8. https://doi.org/10.1603/IPM12006

Karthikeyan, A., Shobhana, V. G., Sudha, M., Raveendran, M., Senthil, N., Pandiyan, M., & Nagarajan, P. (2014). Mungbean yellow mosaic virus (MYMV): A threat to green gram (*Vigna radiata*) production in Asia. *International Journal of Pest Management*, *60*(4), 314–324. https://doi.org/10.1080/09670874.2014.982230

Kasmiyati, S., Kristiani, E. B. E., & Herawati, M. M. (2020). Effect of induced polyploidy on plant growth, chlorophyll and flavonoid content of *Artemisia cina*. *Biosaintifika: Journal of Biology & Biology Education*, *12*(1), 90–96. https://doi.org/10.15294/biosaintifika.v12i1.22548

Kaura, S., & Parle, M. (2017). Anti-ageing activity of moong bean sprouts. *International Journal of Pharmaceutical Sciences and Research*, *8*(10), 4318–4324. https://doi.org/10.13040/IJPSR.0975-8232.8(10).4318-24

Khursheed, S., Raina, A., Laskar, R. A., & Khan, S. (2018). Effect of gamma radiation and EMS on mutation rate: Their effectiveness and efficiency in faba bean (*Vicia faba* L.). *Caryologia*, *71*(4), 397–404. https://doi.org/10.1080/00087114.2018.1485430

Melsen, K., van de Wouw, M., & Contreras, R. (2021). Mutation breeding in ornamentals. *HortScience*, *56*(10): 1154–1165. https://doi.org/10.21273/HORTSCI16001-21

Mishra, G. P., Dikshit, H. K., S. V, R., Tripathi, K., Kumar, R. R., Aski, M., Singh, A., Roy, A., Priti, Kumari, N., Dasgupta, U., Kumar, A., Praveen, S., & Nair, R. M. (2020). Yellow mosaic disease (YMD) of mungbean (*Vigna radiata* (L.) Wilczek): Current status and management opportunities. *Frontiers in Plant Science*, *11*, 918. https://doi.org/10.3389/fpls.2020.00918

Mori, S., Yamane, T., Yahata, M., Shinoda, K., & Murata, N. (2016). Chromosome doubling in *Limonium bellidifolium* (Gouan) dumort. by colchicine treatment of seeds. *Horticulture Journal*, *85*(4), 366–371. https://doi.org/

10.2503/hortj.MI-117

Mustafa, N. S., Liu, F., Odongo, M. R., Wang, X., Cai, X., Zhang, Z., & Wang, K. (2017). Effects of colchicine treatments on chromosome doubling in three diploid cotton species. *Journal of Multidisciplinary Engineering Science and Technology*, *4*(4), 7140–7145. Retrieved from http://www.jmest.org/wp-content/uploads/JMESTN42352173.pdf

Nair, R. M., Pandey, A. K., War, A. R., Hanumantharao, B., Shwe, T., Alam, A. K. M. M., Pratap, A., Malik, S. R., Karimi, R., Mbeyagala, E. K., Douglas, C. A., Rane, J., & Schafleitner, R. (2019). Biotic and abiotic constraints in mungbean production—progress in genetic improvement. *Frontiers in Plant Science*, *10*, 1340. https://doi.org/10.3389/fpls.2019.01340

Niu, L., & Liao, W. (2016). Hydrogen peroxide signaling in plant development and abiotic responses: Crosstalk with nitric oxide and calcium. *Frontiers in Plant Science*, *7*, 230. https://doi.org/10.3389/fpls.2016.00230

Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. A., Miah, G., & Usman, M. (2015). Principle and application of plant mutagenesis in crop improvement: A review. *Biotechnology and Biotechnological Equipment*, *30*(1), 1–16. https://doi.org/10.1080/13102818.2015.1087333

Piasecka, A., Jedrzejczak-Rey, N., & Bednarek, P. (2015). Secondary metabolites in plant innate immunity: Conserved function of divergent chemicals. *New Phytologist*, *206*(3), 948–964. https://doi.org/10.1111/nph.13325

Roychowdhury, R., & Tah, J. (2011). Chemical mutagenic action on seed germination and related agro-metrical traits in M1 Dianthus generation. *Current Botany*, *2*(8), 19–23. Retrieved from https://www.researchgate.net/publication/215757097_Chemical_Mutagenic_Action_on_Seed_Germination_and_Related_Agro-metrical_Traits_in_M1_Dianthus_Generation

Salama, H. S. A., Nawar, A. I., & Khalil, H. E. (2022). Intercropping pattern and N fertilizer schedule affect the performance of additively intercropped maize and forage cowpea in the Mediterranean Region. *Agronomy*, *12*(1), 107. https://doi.org/10.3390/agronomy12010107

Smirnoff, N., & Arnaud, D. (2019). Hydrogen peroxide metabolism and functions in plants. *New Phytologist*, *221*(3), 1197–121. https://doi.org/10.1111/nph.15488

Stec, K., Kordan, B., & Gabryś, B. (2021). Effect of soy leaf flavonoids on pea aphid probing behavior. *Insects*, *12*(8), 756. https://doi.org/10.3390/insects12080756

Susrama, I. G. K., & Pradnyawathi, N. L. M. (2020). Induced *in vivo* mutagenesis using colchicine in hydrogen peroxide on M2 cowpea, yardlong bean and common bean. *International Journal of Biosciences and Biotechnology*, *7*(1), 1–11. https://doi.org/10.24843/ijbb.2019.v07.i01.p01

Susrama, I. G. K., & Wirawan, I. G. P. (2017). Crop improvement through inducing mutagenesis *in vivo* using colchicine on cowpea (*Vigna unguiculata* L. Walp). *International Journal of Biosciences and Biotechnology*, *4*(2), 85–91. https://doi.org/10.24843/ijbb.2017.v04.i02.p02

Talebi, A. B., Talebi, A. B., & Shahrokhifar, B. (2012). Ethyl methane sulphonate (EMS) induced mutagenesis in Malaysian rice (cv. MR219) for lethal dose determination. *American Journal of Plant Sciences*, *3*(12), 1661–1665. https://doi.org/10.4236/ajps.2012.312202

Tammu, R. M., Nuringtyas, T. R., & Daryono, B. S. (2021). Colchicine effects on the ploidy level and morphological characters of Katokkon pepper (*Capsicum annuum* L.) from North Toraja, Indonesia. *Journal of Genetic Engineering and Biotechnology*, *19*(1), 31. https://doi.org/10.1186/s43141-021-00131-4

Tian, Y., Fan, M., Qin, Z., Lv, H., Wang, M., Zhang, Z., Zhou, W., Zhao, N., Li, X., Han, C., Ding, Z., Wang, W., Wang, Z. Y., & Bai, M. Y. (2018). Hydrogen peroxide positively regulates brassinosteroid signaling through oxidation of the BRASSINAZOLE-RESISTANT1 transcription factor. *Nature Communications*, *9*(1), 1063. https://doi.org/10.1038/s41467-018-03463-x

Tsukaya, H. (2013). Does ploidy level directly control cell size? Counterevidence from arabidopsis genetics. *PLoS ONE*, *8*(12), e83729. https://doi.org/10.1371/journal.pone.0083729

Tudi, M., Ruan, H. D., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., & Phung, D. T. (2021). Agriculture development, pesticide application and its impact on the environment. *International Journal of Environmental Research and Public Health*, *18*(3), 1112. https://doi.org/10.3390/ijerph18031112

Wang, Z., Fan, G., Dong, Y., Zhai, X., Deng, M., Zhao, Z., Liu, W., & Cao, Y. (2017). Implications of polyploidy events on the phenotype, microstructure, and proteome of *Paulownia australis*. *PLoS ONE*, *12*(3), e0172633. https://doi.org/10.1371/journal.pone.0172633

Xue, Z., Wang, C., Zhai, L., Yu, W., Chang, H., Kou, X., & Zhou, F. (2016). Bioactive compounds and antioxidant activity of mung bean (*Vigna radiata* L.), soybean (*Glycine max* L.) and black bean (*Phaseolus vulgaris* L.) during the germination process. *Czech Journal of Food Sciences*, *34*(1), 68–78. https://doi.org/10.17221/434/2015-CJFS