**Running title:** Performance, Genetic Variability and Heritability

**Performance, Genetic Variability and Heritability of M1 Generation Mandarin Citrus (*Citrus reticulata* L) Mutants**

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**Novelty statement:**

Improving the traits of local Indonesian mandarin citrus can be done through mutation breeding followed by selection. Selection will be effective if the characteristics, genetic variability, and heritability of the traits of interest in the mutation generation are known. The results showed that mandarin citrus performance were varied and influenced by genotype and radiation dosage. Wide genetic variability and high broad sense heritability in M1 generation of mandarin citrus mutant were found in several of observed traits. Thus, selection for that traits at early generation using a simple selection method may be considered.

**Abstract**

Mandarin citrus (*Citrus reticulata* L) are one of the most popular fruits in Indonesia. Consumers like citrus fruits that have few or no seeds (seedless), easy to peel and has an attractive color. This type of citrus fruit can be obtained through mutation plant breeding followed by selection. The purpose of this study was to examine the performance, determine the genetic variability, and predict the heritability of M1 generation local Indonesian mandarin citrus mutans. Materials irradiated were buds from mandarin citrus cultivars, namely Siam Madu, Sitaya and Tawangmangu. Irradiation dose were used are 0 Gy, 50 Gy and 60 Gy. Variables observed were bud length (cm), bud color, leaf area, leaf color, photosynthetically active radiation (PAR), net photosynthesis, stomatal conductance, and respiration. The results showed that performance of mandarin citrus mutant was varied and influenced by genotype and irradiation dose used. Wide genetic variability was obtained on bud color, leaf area, leaf color and net photosynthesis traits. Except for bud length, all observed traits showed high broad sense heritability values. Based on wide genetic variability and high broad sense heritability values, selection for bud color, leaf area, leaf color and net photosynthesis traits can be carried out at early generation using a simple selection method.

**Keywords:** mandarin citrus, mutations, genetic variability, broad sense heritability

**Introduction**

Citrus is one of fruit that is widely consumed and there are many local cultivars in Indonesia. National citrus production in 2021 is 2.51 million tons, decrease 7.67% from 2020. Meanwhile in the same year citrus household consumption reached 1.15 million tons in 2021, increased by 29.95% from 2020 consumption (BPS, 2022a). Citrus production in 2021 will be mostly contributed by mandarin oranges (*Citrus reticulata* L). In 2021 mandarin citrus will contribute 2.41 million tons of total national production (BPS, 2022b).

Indonesia has several popular local mandarin citruses, including Siam Madu, Sitaya and Tawangmangu cultivars. One of local mandarin citrus weaknesses is have a lot of seeds so they are not liked by consumers. Criteria for citrus fruits favored by consumers and the global market are having few or no seeds (seedless), easy to peel and has an attractive color (Karyanti et al., 2015). Thus improving the properties of oranges aims to produce quality citrus fruits (in terms of size, sugar and acidity balance, juice yield, and seedless), which are healthy and rich in antioxidant compounds, tolerant or resistant to different abiotic and biotic threats, as well as high productivity (Salonia et al., 2020).

Improving seedless citrus can be done through plant breeding programs. Conventional breeding of citrus is slow and difficult due to its complex reproductive biology such as slow fruit growth and ripening, heterozygosity, apomixis, polyembryony, parthenocarpy, incompatibility, and long juvenile phase (Febres et al., 2011; Agisimanto et al., 2016; Kim et al., 2020; Ollitrault et al., 2021). Another alternative method that can be used to solve this problem is plant mutation. Mutations are changes in gene composition, which are reported to improve plant characteristics (Herwibawa et al., 2014).

Mutational breeding has shown enormous potential in citrus crops for improvement especially in economically important horticultural traits (Mustafa et al., 2021). Mutation induction has been widely used in citrus to develop varieties with better quality and resistance or tolerance to biotic and abiotic stresses (Kamatyanatt et al., 2021). Mutation induction techniques such as radiation or chemical mutagens are good tools to increase variability within plant species because spontaneous mutations occur with a very low frequency. (Sutarto et al., 2009). Gamma rays are the most widely used as mutagen irradiation in plant mutation breeding (Purba et al., 2021; Habibullah et al., 2022). Mutation induction with gamma rays has been proven effective in several citrus species (Eun and Kim, 2022). Irradiation of woody plants with gamma rays can produce a higher frequency of mutations, which leads to the creation of new variants compared to the parent varieties. (Rattanpal et al., 2019).

Gamma ray irradiation has been shown to be effective in increasing citrus diversity (Arisah and Mariana, 2018; Eun and Kim, 2022). Gamma ray irradiation can produce high mutations to create new genetic variability that is different from the wild type oranges (Rattanpal et al., 2019). Mutation breeding has proven to be a powerful tool for increasing the spectrum of genetic variability (Akhtar et al., 2015; Patil and Lokesha, 2018). Genetic variability is a prerequisite for any initiation of crop improvement programs and the application of appropriate selection techniques (Chowdhury et al., 2023). Studying genetic variability with reference to genetic parameters such as genetic variability coefficient and heritability reveal the inheritance of quantitative and qualitative parameters of fruit to design citrus plant breeding strategies (Singh et al., 2022).

Genetic variability coefficient is a value that describes the genetic variability of a trait in a population. Genetic variability coefficient gives a better picture of genetic variability (Sudeepthi et al., 2020). Genetic variability coefficient can be divided into two, namely phenotypic variability coefficient (PCV) and genotypic variability coefficient (GCV) (Singh et al., 2011; Riyanto et al., 2021). PCV and GCV are useful in detecting the level of variabilities of a particular trait (Chavan et al., 2020).

Heritability shows a trait is more controlled by genetic factors or environmental factors so that it can be seen to what extent the traits is inheritance on to next offspring (Kartahadimaja et al., 2021). Heritability is the proportion of genetic variance to phenotypic variance (Khomphet et al., 2022). Heritability of a trait is very important in determining the response to selection (Riyanto et al., 2021) and this illustrates the effectiveness of genotypic selection based on phenotypic performance (Shah et al., 2018).

Mutation induction can increase the genetic diversity of Siam Madu, Sitaya and Tawangmangu mandarin citrus cultivars. In this mandarin citrus mutant population, information of genetic variability and heritability is required so that selection becomes efficient. The purpose of this study was to examine the performance, determine the genetic variability, and predict the heritability of M1 generation local Indonesian mandarin citrus mutans.

**Materials and Methods**

**Experimental location**

Radiation induction was carried out at the National Nuclear Energy Agency of Indonesia (BATAN). The experiment was carried out in citrus land in Kembanglimus Village, Borobudur District, Magelang Regency. The research was conducted on form March to September 2022.

**Experimental materials**

Citrus plants grafted with citrus buds that have been irradiated at doses of 0.50 Gy and 60 Gy were used in this study. The irradiated buds were mandarin citrus cultivar i.e., Siam Madu, Sitaya and Tawangmangu.

**Experimental design**

The experimental design was a factorial randomized block design with three replications. There are two factors tested, namely gamma ray irradiation dose and citrus genotype. The gamma irradiation doses tested were 0 Gy, 50 Gy and 60 Gy. Citrus genotype consisted of Siam Madu, Sitaya and Tawangmangu cultivars. Mandarin citrus buds cv. Siam Madu, Sitaya and Tawangmangu with criteria bud length 20-25 cm, there are 3-8 buds, taken from branches that are protected from pests and diseases irradiated using gamma rays with doses of 0 Gy, 50 Gy and 60 Gy. The irradiated buds were grafted onto a five-year-old mandarin citrus tree by means of bud grafting. Data were collected on the buds at 12 weeks after grafting.

**Data collection and analysis**

Variables observed were bud length, bud color, leaf area, leaf color, photosynthetically active radiation (PAR), net photosynthesis, stomatal conductance, and respiration. To determine the performance of observed traits data obtained were analyzed for variance at an error level of α 5% (Table 1). If there is a significancy, continue with Duncan's Multiple Range Test at an error level of 5%. Estimate of genetic parameters was calculated followings.

1. Estimation of phenotypic variance genotypic variance , and environmental variance

The phenotypic variance, genotypic variance and environmental variance were estimated based on the estimation of the mean square of analysis of variance (Table 1), calculated in the following (Annicchiarico, 2002; Jambormias, 2014).

where, = environmental variance, = interaction of radiation dose x genotype variance, = genotypic variance dan = phenotypic variance.

1. Estimation of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV)

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated following (Singh and Chaudhary, 1979).

where, = phenotypic variance, = genotypic variance and = population mean value.

PCV and GCV are grouped into high (coefficient value more than 20%), moderate (coefficient value between 10% - 20%) and low (coefficient value less than 10%) (Sivasubramanian and Menon, 1973).

1. Estimation of broad sense heritability (h2bs)

Broad meaning heritability (h2bs) were calculated following (Roy, 2000).

where, = phenotypic variance, = genotypic variance and = population mean value.

Broad sense heritability is categorized into high (value greater than 50%), moderate (between 50% and 20%) and low (value less than 20%) (Stansfield, 1991).

**Results**

1. Performance of mandarin citrus mutants at several gamma ray irradiation doses

Analysis of variance result are presented in Table 2. Analysis showed that radiation dose caused significant differences in bud length, bud color, leaf color and net photosynthesis. Genotype led to marked differences in all observed traits. The interaction between radiation dose and genotype was significantly different in bud length, bud color, leaf color and net photosynthesis.

Table 3 shows an irradiation dose of 60 Gy decreased bud length of Siam Madu and Tawangmangu cultivar, but it is increased in Sitaya cultivar. Irradiation dose affected bud color, leaf color, and net photosynthesis only in Siam Madu cultivar but not in Sitaya and Tawangmangu cultivar. In Siam Madu cultivar, an irradiation dose of 50Gy caused an increase in leaf color and net photosynthesis, but these traits decreased in irradiation dose of 60Gy.

1. Genetic variability and broad sense heritability of M1 Generation Mandarin Citrus Mutants

Analysis of variance showed that there was variation in genotypes factor (Table 2). Table 4. shows the phenotypic variance of all observed variables which is greater than the genotypic variance. this result was followed by a PCV which was greater than the GCV. GCV values ​​for the observed properties ranged from 7.29 – 68.51%. bud color, leaf area, leaf color and net photosynthesis showed KKG values ​​above 20%, respectively 20.86%, 68.51%, 28.98%, and 29.93%.

Broad sense heritability estimation value of observed trait ranged from 5.28% - 91.00% (Table 4.). Traits that show a value above 50% are bud color (76.71%), leaf area (90.25%), leaf color (88.94%), net photosynthesis (91.00%), PAR (77.57%), respiration (56.81%) and stomatal conductance (87.74%).

**Discussion**

1. Performance of mandarin citrus mutants at several gamma ray irradiation doses

Analysis of variance in Table 2. shows that bud length, bud color, leaf color and net photosynthesis were affected by the interaction between irradiation doses and mandarin citrus cultivars. Irradiation dose affected bud color, leaf color and net photosynthesis only in the Siam Madu cultivar but did not affect Sitaya and Tawangmangu cultivars. The results of this study indicate that the success of gamma ray irradiation is influenced by level of irradiation dose and genotype. The rate of mutation induction can be determined by critical factors such as gamma ray irradiation dose and genotype used (Sutarto et al., 2009; Pérez-Jiménez et al., 2020; Yarar et al., 2022).

The results showed that in Siam Madu cultivar the irradiation dose at 50Gy caused an increase in leaf color and photosynthesis (PN), but both traits decreased at the irradiation dose of 60Gy. This means that the irradiation dose of 50Gy is effective for Siam Madu cultivar. These results agree with other studies that the LD50 in bud was obtained at a dose of 50Gy gamma irradiation (Latado et al., 2012; Pérez-Jiménez et al., 2020).

1. Genetic variability and broad sense heritability of M1 Generation Mandarin Citrus Mutants

The results showed that there was variance in the observed genotypes. Variance can be separated into phenotypic variance, genotypic variance and environmental variance (Yani et al., 2018). So that information about the contribution of genetic variability to the total observed variance can be known.

In this study, PCV value was greater than GCV value for all observed traits. PCV reflects the influence of environmental factors and GCV describes the influence of genetic factors on a trait (Kishore *dkk.* 2015; Chozin *dkk.* 2017). PCV which is greater than GCV indicates that there is an influence of the environment on the expression of the observed trait (Choudhary *dkk.* 2018b; Sudeepthi *dkk.* 2020).

The small difference between PCV and GCV indicates that genetic factors are more influential than environmental factors as shown by bud color, leaf area, net photosynthesis, respiration, and stomatal conductance. Selection based on the phenotype for traits that have almost the same GCV and PCV values ​​will be effective for improving these traits (Rani et al., 2016). Therefore, selection of citrus mandarin mutants can be based on bud color, leaf area, net photosynthesis, respiration, and stomatal conductance.

Genotypic coefficient of variation which is above 20% is categorized as high value indicates wide genetic variability (Sivasubramanian and Menon, 1973). The results of this study showed that the GCV values ​​of bud color, leaf area, leaf color, and net photosynthesis were above 20%, reflecting that these traits have a wide genetic variability. Wide genetic variability in the population provides flexibility in selection in order to assemble a new variety with traits suitable for the purpose (Bornare et al., 2014).

Heritability broad sense value of bud color, leaf area, leaf color, net photosynthesis, PAR, respiration, and stomatal conductance traits above 50%. Heritability values ​​above 50% are categorized as high heritability (Stansfield, 1991). This means that bud color, leaf area, leaf color, net photosynthesis PAR, respiration and conductance traits have high heritability values.

Traits with high heritability values ​​indicate genetic factors are more influential than environmental factors on performance of these traits (Riyanto et al., 2023a). Traits with high heritability values ​​can be improved using simple selection methods (Raghavendra and Hittalmani, 2016). In addition, selection of traits with high heritability values ​​can be carried out in the early generations because dominant genetic factors influence plant phenotypes (Lestari et al., 2015) thus accelerating the cultivar development process (Riyanto et al., 2023b). Therefore, based on the heritability value, the selection of buds color, leaf area, leaf color, net photosynthesis, PAR, respiration, and stomatal conductance traits can be carried out at the beginning of the generation using a simple selection method.

Selection of a trait will be effective if genetic variability and heritability are combined. In this study, based on genetic variability and heritability selection for bud color, leaf area, leaf color and net photosynthesis traits can be carried out at early generation using a simple selection method.

**Conclusion**

The results showed that performance of mandarin citrus mutant was varied and influenced by genotype and irradiation dose used. Wide genetic variability was obtained on bud color, leaf area, leaf color and net photosynthesis traits. Except for bud length, all observed traits showed high broad sense heritability values. Based on wide genetic variability and high broad sense heritability values, selection for bud color, leaf area, leaf color and net photosynthesis traits at early generation using a simple selection method may be considered.

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**Author Contributions**

All authors participated in the elaboration, discussion and writing of this paper and they are responsible for the content of the manuscript.

**Conflict of Interest**

All authors declare no conflict of interest

**Data Availability**

Data presented in this study will be available on a fair request to the corresponding author

**Ethics Approval**

Not applicable to this paper

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**Table 1.** Model of analysis of irradiation dose, genotype, and interaction of irradiation dose x genotype of M1 generation mandarin citrus mutant traits

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Source of  variation | Df | Sum square | Mean Square | Variance  component |
| Block | r-1 | SSr | M5 |  |
| Irradiation dose (D) | d-1 | SSd | M4 |  |
| Genotype (G) | g-1 | SSg | M3 |  |
| D x G interaction | (d-1) (g-1) | SSdg | M2 |  |
| Error | (r-1) (ab-1) | SSe | M1 |  |
| Total | rdg-1 |  |  |  |

**Table 2.** Means square of analysis of anova of M1 generation mandarin citrus mutant traits

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Traits | Irradiation dose (D) | | Genotype (G) | | D x G Interaction | |
| Bud length | 1071.68 | \* | 1159.48 | \* | 1081.76 | \* |
| Bud color | 3.06 | \* | 7.91 | \* | 1.52 | \* |
| Leaf area | 81.04 |  | 280.22 | \* | 60.95 |  |
| Leaf color | 1.97 | \* | 12.71 | \* | 1.18 | \* |
| PAR | 131434.45 |  | 707802.65 | \* | 139516.97 |  |
| Net photosynthesis | 17.59 | \* | 144.19 | \* | 10.42 | \* |
| Respiration | 0.06 |  | 2.10 | \* | 0.86 |  |
| Stomatal conductance | 1855.26 |  | 2352.90 | \* | 376.90 |  |

Remark: \* = significantly different at α = 5%.

**Table 3.** Interaction effect of irradiation dose and genotype of M1 generation mandarin citrus mutant traits

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Genotype | Irradiation dose (Gy) | Bud length (cm) | | Bud color | | Leaf color | | Net photosynthesis | |
| Siam Madu | 0 | 51.20 | a | 3.15 | a | 2.17 | b | 6.67 | b |
|  | 50 | 64.37 | a | 3.32 | a | 3.25 | a | 12.07 | a |
|  | 60 | 16.49 | b | 1.21 | b | 1.22 | b | 6.87 | b |
| Sitaya | 0 | 25.98 | b | 4.42 | a | 3.79 | a | 9.44 | a |
|  | 50 | 35.99 | ab | 3.78 | a | 3.92 | a | 10.35 | a |
|  | 60 | 46.51 | a | 3.83 | a | 3.65 | a | 9.74 | a |
| Tawangmagu | 0 | 21.21 | ab | 4.05 | a | 4.33 | a | 14.85 | a |
|  | 50 | 35.27 | a | 4.00 | a | 4.12 | a | 15.22 | a |
|  | 60 | 16.97 | b | 3.73 | a | 4.00 | a | 15.19 | a |

Remark: \* numbers followed by the same letter for the same factor and traits were not significantly different in the DMRT at α = 5%.

**Table 4.** Phenotypic variance, genotypic variance, environmental variance, phenotypic coefficient of variation, genotypic coefficient of variation and broad sense heritability of M1 generation mandarin citrus mutant traits

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Traits | σ2p | σ2g | σ2e | PCV (%) | GCV (%) | h2bs (%) |
| Bud length | 122,75 | 6,48 | 141,35 | 31,76 | 7,29 | 5,28 |
| Bud color | 0,69 | 0,53 | 0,27 | 23,82 | 20,86 | 76,71 |
| Leaf area | 3,15 | 2,84 | 1,53 | 72,12 | 68,51 | 90,25 |
| Leaf color | 1,08 | 0,96 | 0,40 | 30,73 | 28,98 | 88,94 |
| PAR | 12,25 | 11,15 | 1,99 | 31,37 | 29,93 | 91,00 |
| Net photosynthesis | 61053,35 | 47357,14 | 65004,36 | 15,68 | 13,81 | 77,57 |
| Respiration | 0,18 | 0,10 | 0,59 | 13,92 | 10,49 | 56,81 |
| Stomatal conductance | 187,69 | 164,67 | 678,89 | 13,90 | 13,02 | 87,74 |

remark: = phenotypic variance, = genotypic variance, = environmental variance, PCV = phenotypic coefficient of variation, GCV = genotypic coefficient of variation and h2bs = broad sense heritability.