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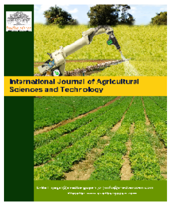

Research Paper

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# Rojolele: A Premium Aromatic Rice Variety in Indonesia

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## Abstract

Rojolele is an Indonesian traditional rice variety from Klaten (Central Java) that classified as tropical japonica and low land rice which become a premium rice variety and have higher price in the market due to a delicious taste and a fragrance aroma. The agronomic characteristics of Rojolele are having high plant stature with sturdy stems and upright plant shape, thick and rough leaves, strong and deeper root systems, long panicle length, and long duration of life. Rojolele rice cultivation is facing important challenges from drought, diseases, long duration, and lodging. Thus, breeding Rojolele cultivars with resistance to drought, diseases, lodging, and short duration is the major focus for Rojolele rice improvement. Indonesian farmer have learned to successfully cultivate Rojolele by applying practical skills, leading to increase the rice productivity. Molecular breeding program, including Quantitative Trait Loci (QTL), Genome-Wide Association Study (GWAS), genomic selection, and genome editing can be applied to improve Rojolele characteristics. In this review, important agronomic and quality traits, intensification system for irrigation and pest control, mutation breeding, transgenic lines, and also future perspectives for Rojolele research were presented. Rojolele is useful for rice breeding program in order to guarantee the food security to overcome increasing population and climate change.

***Keywords:*** *Rojolele, Aromatic rice, Intensification system, Mutation breeding, Transgenic lines*



## 1. Introduction

In Indonesia, rice is a primary staple food for around 250 million people and also become an economic source for majority communities in rural areas. Annually, Indonesia needs around 50 million tons of rice with annual per capita rice consumption reaching 140-150 kg. Indonesia also the third largest rice producer in the world after China and India. Recently, rice production in Indonesia is decreasing 26% due to the farmers still using conventional systems in rice cultivation leading inefficiency of using water and fertilizer. This rice production shortages negatively affect economic, social, and political aspects (Hariyono and Isnawan, 2018). In order to stabilize the rice prices, the government has to develop effective methods to increase the rice productivity.

Indonesian archipelago rich in biodiversity of traditional rice germplasm as rice genetic resources because of location in the equator zone with tropical climate and two seasons (dry and wet seasons) which make Indonesia become a perfect

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place to cultivate rice and known as an agrarian country. Every region in Indonesia has more than one traditional rice varieties that have been cultivated for hundreds of years with delicious taste and has special aroma according to the rice consumers in the local community, more resistant to abiotic and biotic stresses, adapts well to the location where the varieties originates, and also more adaptable to climate change compared to introduced rice varieties. Many traditional rice varieties have been identified showing resistance to abiotic stresses, such as salt, drought, heat, cold, Fe toxicity, and Al toxicity. Several traditional rice varieties also showing resistance to biotic stresses, including tungro, rice stripe virus, neck blast, leaf blast, brown planthopper, and rice gall midge (Sitaresmi *et al.*, 2013). Meanwhile, several weakness of the traditional rice varieties including late maturity, long growth duration, low tiller number, susceptible to lodge, and low grain yield. About 3,800 traditional rice germplasms are registered by Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (Dewi *et al.*, 2020). For example, Rojolele from Klaten (Central Java), Pandan Wangi from Cianjur (West Java), Jembar from West Java, Kewal from Banten (West Java), Kuriak Kusuik from West Sumatra, Barak Cenana from Tabanan (Bali), Siam Datu from South Kalimantan, etc. These traditional rice varieties are useful for rice breeding program in order to guarantee the food security to overcome increasing population and climate change.

Most of Indonesian traditional rice varieties belong to tropical japonica (known as *javanica*) subspecies which commonly tall stature that make them susceptible to lodge, late maturity, low productive tiller number, deep rooting system, wide leaves, photoperiod insensitivity, long panicles, round seed shape with long awn at the end of the grain, intermediates amylose content, and low yield potential (Sitaresmi *et al.*, 2013; Dwiningsih *et al.*, 2019; Hairmansis *et al.*, 2015; Irawan and Purbayanti, 2008; Siwi and Harahap, 1978). Generally, the traditional rice varieties cultivated in unfavorable field conditions with no inputs of chemical fertilizers and pesticides, such as acidic dry land, high salinity of swampy land, dry land in hilly areas, and flooded area. Farmers still using on their own seeds for the next planting season, so the level of the seed purity is very low, that negatively affects the rice production. Because of the low level of seed purity, the performance of traditional rice varieties become so diverse in heading days, plant height, tiller number that leading to the low grain yield and grain quality. In order to improve the level of seed purity and to increase rice productivity of traditional rice varieties, the government has been released purified seeds of eleven traditional rice varieties, such as Rojolele, Pandan Wangi, Anak Daro, Kuriek Kusuik, Junjung, Caredek Merah, Lampai Kuning, Siam Mutiara, Siam Saba, Cekow, and Karya (Dewi *et al.*, 2020). Recently, most of these traditional rice varieties are still cultivating in certain regions in Indonesia.

In this review, description related to Rojolele as the most popular traditional rice variety in Indonesia are provided. Important agronomic and quality traits, intensification system for irrigation and pest control, mutation breeding, transgenic lines, and also future perspectives for Rojolele research were presented. The premium agronomic, grain and cooking quality traits of Rojolele will participate in overcoming the decreasing rice productivity in Indonesia by reducing the cost of rice cultivation which related to less application of chemical fertilizers and pesticides.

## 2. Agronomic Characteristics of Rojolele

Rojolele is an indigenous Indonesian rice variety from Delanggu village, Klaten city, Central Java province, Indonesia which become a premium rice variety and have higher price in the market due to a delicious taste and a fragrance aroma. Based on the taxonomic status of aromatic rice, Rojolele belongs to group VI along with Mentik Wangi (Indonesia), Xiang Keng 3 (China), Sukanandi (Indonesia), Milfore (6) 2 (Philippines), Azucena (Philippines), and Milagrosa (Philippines) (Khush, 2000). Rojolele is classified in tropical japonica (*javanica*) subspecies and lowland rice cultivar (Rachmawati *et al.*, 2004). The cultivated area of Rojolele is reaching 10,000 ha in three different area of Java island, including Central Java, West Java, and East Java.

The agronomic characteristics of Rojolele are having high plant stature with sturdy stems and upright plant shape, thick and rough leaves, strong and deeper root systems, long panicle length, and long duration of life (Table 1). Because of this long duration of life reaching 150 days after sowing (das), the rice productivity of Rojolele is high which can reach 8-10 ton/ha. The regular rice varieties only take 120 days for harvesting (Hariyono and Isnawan, 2018; Ramadhan *et al.*, 2022; Dwiningsih *et al.*, 2021; Ge *et al.*, 2022). Flowering time for Rojolele is around 116 das and the grain maturity time reaching 150 das. Rojolele showing superior agronomic characteristics compared to the other popular rice varieties in Indonesia such as Ciherang, Memberamo, and Inpari19 especially for the plant height, number of tillers, leaf area, fresh weight, dry weight, productive tiller number, root length, panicle length, and grain weight per plant. This superiority is influenced by the longer lifespan of Rojolele which enable to support the optimum growth of the plants, such as optimum photosynthetic activity that related to the grain filling process leading to the higher grain yield of Rojolele. Meanwhile, Rojolele has higher unfilled grain number, low productive tiller number, and less total root number compared

**Table 1: Agronomic Characteristics of Rojolele**

| Agronomic Traits | Rojolele |
|---|---|
| Plant height | 165 cm |
| Total number of tillers | 18 |
| Productive tiller number | 9 |
| Total leaf area | 535 $cm^2$ |
| Specific leaf weight (SLW) | 0.0081 $g/cm^2$/week |
| Net assimilation rate (NAR) | 0.0116 $g/cm^2$/week |
| Relative growth rate (RGR) | 0.341 g/g/week |
| Crop growth rate (CGR) | 0.0082 $g/cm^2$/week |
| Fresh weight of plants | 312 g |
| Dry weight of plants | 60 g |
| Root length | 56.36 cm |
| Total root number | 29 |
| Panicle length | 40 cm |
| Grain length | 7 mm |
| Grain weight per plant | 43.31 g |
| Lightness value of grain | 74 |
| Unfilled grain | 12.40% |
| Harvest index | 0.8 |
| Duration of life | 150 days after sowing |

*Source: Hariyono and Isnawan (2018)*

to Ciherang, Memberamo, and Inpari19. Furthermore, Rojolele has no significantly different in leaf area, harvest index, Net Assimilation Rate (NAR), Relative Growth Rate (RGR), Crop Growth Rate (CGR), and Specific Leaf Weight (SLW) with Ciherang, Memberamo, and Inpari19 (Hariyono and Isnawan, 2018).

Rice grains of Rojolele belong to high-quality rice that have long and slender grains with bright white or white milk color. Physical grain quality of Rojolele, including the size, shape, and grain color from three different regions in Central Java, such as Karanganom, Polanharjo, and Banyudono are not significantly different. On the other hand, Rojolele is susceptible to several diseases, such as yellow stem borer (*Scirpophaga incertulas*). Rojolele also sensitive to drought stress condition even in the low level of drought –0.03 MPa. The aromatic rice cultivars like Rojolele are more sensitive to drought stress condition compared to the non-aromatic rice cultivars, due to the total proline content in the rice aromatic cultivar is lower on the low level of drought stress condition (Dwiningsih, 2020; Joshi *et al.*, 2011). Moreover, Basu *et al*. (2010), also described that under drought stress conditions the aromatic rice cultivars increase on peroxide ($H_2O_2$) level, lipoxygenase (LOX) and malondialdehyde (MDA) activities, and accumulating carbonylated protein derivatives. The price of Rojolele is more expensive compared to the others rice varieties due to the high grain quality, long duration planting, and the complexity of pest management in the post-harvest time. Thus, most farmers refuse to plant Rojolele in their field.

## 3. Cooking Quality, Fragrance Aroma and Interaction with Environments

The high-quality grain of Rojolele has long and slender grains with intermediate gelatinization temperature, intermediate amylose content, and scored as strongly fragrance aroma. After cooking process, the grain has a soft texture, delicious taste along with high elongation ratio (Dwiningsih *et al.*, 2020). The name of Rojolele is derived from the good texture, taste, and fragrance aroma that meet the rice consumer's demand, which rice consumers found Rojolele to be the king among the rice varieties in Indonesia. Since the rice grain of Rojolele is fragrance, several insects and birds such as planthoppers and sparrows were attracted to eat the grains which make farmers to do more works to manage the fields.

**Table 2: Nutrient Content of Rojolele**

| Nutrient Content | Rojolele |
|---|---|
| Carbohydrate | 93% |
| Amylose | 30% |
| Protein | 6% |
| Fat | 1% |
| Ash | 0.40% |

*Source: Setyawan et al. (2017)*

Nutrient content of Rojolele rice, such as carbohydrate, amylose, protein, fat, and ash have been identified (Table 2) (Setyawan *et al.*, 2017). Rojolele contains higher amylose than mega-variety IR-64. Geographical location influence the nutrient content of Rojolele. For example, carbohydrate, amylose and protein contents were significantly different in three regions of Karanganom, Polanharjo, and Banyudono.

Rice market for Rojolele as aromatic rice is higher than non-aromatic rice. The fragrance aroma of cooked rice comes from a mixture of around 114 volatile compounds, such as 2-acetyl-1-pyrroline (2AP). Naturally, 2AP is also identified in pandan leaves (*Pandanus amaryllifolius*) and associated with the flavor of corn tortillas, popcorn, green tea, wine, mung bean, cheese, baguettes, and ham (Chen, *etal.*, 2008; Maqsood *et al.*, 2022). The fragrance aroma of jasmine and basmati rice also correlated to 2AP. This 2AP is identified in all plants of aromatic rice varieties except the root part (Buttery *et al.*, 1983). The genes which controlling fragrance aroma of rice is a single recessive gene (fgr) on chromosome 8 which encoding betaine aldehyde dehydrogenase (BADH2) (Jin *etal.*, 2003; Bradbury *et al.*, 2005). Two Restriction Fragment Length Polymorphism (RFLP) markers, RG1 and RG28 linked to fgr gene were identified (Cho *et al.*, 1998). The concentration of 2AP in aromatic rice is influenced by the environmental conditions. Under drought stress condition, the concentration of 2AP is higher compared to the normal condition (Dwiningsih *et al.*, 2021).

## 4. Intensification System of Rojolele Cultivation

In order to improve the efficiency of water management in Rojolele cultivation, two irrigation system were applied. These irrigation systems were intermittent irrigation system known as System of Rice Intensification (SRI) and conventional method with continuous flooding (Hariyono and Isnawan, 2018). The parameters observed were morphological, physiological, and grain yield traits (Table 3). Most of the farmers in Indonesia are still using conventional irrigation system with continuous flooding for Rojolele cultivation. Thus, the use of water become inefficient leading to the decreasing of oxygen content in the soil which cause less optimal of root growth and photosynthetic activity. Finally, decrease the productive tiller number and grain yield.

Intermittent irrigation with intensive system can save water up to 40%, less fertilizer by 50%, and increase rice productivity up to 78% compared to the conventional irrigation (Dwiningsih *et al.*, 2021; Sato and Uphoff, 2006; Sitrarasi *et al.*, 2022). In SRI cultivation, the water is not continuously supply, water just supply in a periodic time so the plants can absorb water effectively and leading to increase the root growth, productive tiller numbers, and grain yield. The results of SRI method in Rojolele cultivation showed that intermittent irrigation application affected the morphological, physiological, and grain yield characteristics. SRI method significantly affected the fresh and dry weight of plant, productive tiller number, root length, and grain weight per plant compared to the conventional irrigation (Table 3). Meanwhile, SRI method showed non-significantly affected the plant height, total number of tillers, leaf area, panicle length, harvest index, nNt Assimilation Rate (NAR), Relative Growth Rate (RGR), Crop Growth Rate (CGR), Specific Leaf Weight (SLW), and unfilled grain percentage compared to the conventional irrigation (Table 3). Fresh weight of plants in SRI method showed relatively higher than in continuous flooding irrigation due to aerobic soil condition triggering

**Table 3: Morphological, Physiological, and Grain Yield Characteristics of Rojolele Under Conventional and Intermittent Irrigation**

| Traits | Conventional Irrigation | Intermittent Irrigation (SRI) |
|---|---|---|
| Plant height (cm) | 92.96[a] | 93.08 [a] |
| Number of tillers | 14.56 [a] | 16.02 [a] |
| Fresh weight of plant (g) | 189.68 [a] | 266.02 [b] |
| Dry weight of plant (g) | 61.84 [a] | 59.84 [b] |
| Productive tiller number | 9.45 [a] | 8.75 [b] |
| Root length (cm) | 51.52 [a] | 56.36 [b] |
| Leaf area ($cm^2$) | 534.35 [a] | 591.02 [a] |
| Panicle length (cm) | 39.37 [a] | 41.13 [a] |
| Harvest index | 0.89 [a] | 0.90 [a] |
| Net Assimilation Rate (NAR) ($g/cm^2$/week) | 0.0101[a] | 0.0113 [a] |
| Relative Growth Rate (RGR) (g/g/week) | 0.288 [a] | 0.329 [a] |
| Crop Growth Rate (CGR) ($g/cm^2$/week) | 0.0077 [a] | 0.0086 [a] |
| Specific Leaf Weight (SLW) ($g/cm^2$/week) | 0.0081 [a] | 0.0078 [a] |
| Grain weight per plant (g) | 41.31 [a] | 44.25 [b] |
| Unfilled grain (%) | 11.01 [a] | 12.40 [a] |

**Note:** * Means followed by the same letters in a column are not significantly different ($p \leq 0.05$).

root growth and leading to increase the photosynthetic activity, and finally increase the productive tiller numbers and grain yield. Thus, the application of SRI method in Rojolele cultivation is expected to increase profit for the farmers.

## 5. Innovative Technology for Pest Control in Rojolele Cultivation

Fragrance aroma of Rojolele attracts sparrow and planthoppers to eat the grains lead to decrease the farmer's profit. Due to this pest issue, most of the farmers refuse to cultivate Rojolele in their rice fields. Technological assistance is needed to control the pest attacks. Because of the environmental, economic, and health issues, it is important not to apply pesticides to control the pests. Innovative technology of pest control by using ultraviolet (UV) light and ultrasonic sound (US), powered by solar panels can be applied and provide environmental, economic, and social benefits (Ramadhan *etal.*, 2022; Dwiningsih *et al.*, 2022). Due to the abundant sunlight in Indonesia, solar panels are suitable. This UV-US technology only use a low voltage energy and safe for humans because ultrasonic sound cannot be heard by human. The short wave length of ultraviolet light attracts insects, such as planthoppers and they can be trapped in specials vials. The ultrasonic frequency dispels the sparrows. Sustainable agriculture can be achieved by using this UV-US technology for pest control.

Almost all the farmers gave positive reaction to UV-US technology for pest control in their rice fields because the effectivity. Farmers did not need to buy the pesticides and rent the nets to control the pests. By installing this UV-US technology, the $CO_2$ emission also reduce up to 13,155 kg for a month. The unit number of UV-US equipment that install in the rice field also influence the effectivity. Based on the study, instalment of five unit UV-US equipment showed the optimum results of controlling the pests and produce maximum grain yield.

## 6. Mutation Breeding of Rojolele

Mutation breeding of Indonesian local rice varieties, including Rojolele has been approved by the government because of the effectivity and safety for human and environment. The mutation breeding technique has been used for more than

50 years in 44 crops, including rice and many mutated crop cultivars have been released (Bashir *et al.*, 2021; Shu, 2009). A total of 443 rice cultivars have been developed by mutation technique (Dwiningsih and Alkahtani, 2022; Kharkwal and Shu, 2009). Recently, several mutation techniques have been developed and commonly used in rice breeding program. This method is only change the desired traits to increase its economic value without altering preferred traits that already present in the Rojolele rice variety (Dewi *et al.*, 2020; Ali *et al.*, 2021). Rojolele has delicious taste and good aroma, but have some weakness, such as sensitive to drought stress condition, susceptible to lodge, late maturity, and susceptible to yellow stem borer. Mutation breeding by using mutagenic agents, such as physical and chemical mutagen are involved in the development of Rojolele rice variety with superior traits by recombination of alleles on homologous

**Table 4: Performances in Mutation (M1) Generation of Rojolele Under Drought Stress Condition**

| Mutagen | Plant Height (cm) | Root Length (cm) | Leaf Area ($cm^2$) | Culm Diameter (mm) | Number of Tillers |
|---|---|---|---|---|---|
| Wild type | $12.88^{c}$ | $2.83^{e}$ | $5.36^{d}$ | $4.06^{abcd}$ | $1.00^{f}$ |
| γ100Gy | $35.84^{abc}$ | $5.51^{bcde}$ | $14.67^{bcd}$ | $3.95^{abcd}$ | $1.90^{ef}$ |
| γ150Gy | $50.39^{abc}$ | $13.71^{abcde}$ | $15.68^{bcd}$ | $5.47^{ab}$ | $4.20^{abcdef}$ |
| SA2H | $82.05^{abc}$ | $16.84^{abcde}$ | $37.97^{abcd}$ | $4.90^{abcd}$ | $4.80^{abcdef}$ |
| SA6H | $27.33^{bc}$ | $5.87^{bcde}$ | $10.65^{bcd}$ | $4.26^{abcd}$ | $3.60^{bcdef}$ |
| γ100Gy+ SA2H | $80.65^{abc}$ | $13.21^{abcde}$ | $27.38^{bcd}$ | $4.74^{abcd}$ | $4.70^{abcdef}$ |
| γ100Gy+ SA6H | $29.76^{bc}$ | $6.12^{bcde}$ | $11.82^{bcd}$ | $4.52^{abcd}$ | $1.70^{ef}$ |
| γ150Gy+ SA2H | $27.30^{bc}$ | $5.26^{cde}$ | $9.64^{cd}$ | $4.84^{abcd}$ | $2.20^{ef}$ |
| γ150Gy+ SA6H | $58.75^{abc}$ | $11.77^{abcde}$ | $22.37^{bcd}$ | $5.45^{abc}$ | $4.40^{abcdef}$ |

**Note:** * Means followed by the same letters in a column are not significantly different ($p \leq 0.05$); * Means followed by the different letters in a column are significantly different ($p \leq 0.05$).

*Source: Herwibawa et al. (2019)*

**Table 5: Reproductive Performances in Mutation (M1) Generation of Rojolele Under Drought Stress Condition**

| Mutagen | Number of Productive Tiller | Number of Seeds per Panicle | Filled Seed Percentage (%) | 1000 Seeds Grain Weight (g) | Harvest Index |
|---|---|---|---|---|---|
| Wild type | $0.00^{f}$ | $0.00^{d}$ | $0.00^{e}$ | $0.00^{d}$ | $0.000^{b}$ |
| γ100Gy | $0.70^{def}$ | $12.40^{d}$ | $7.22^{cde}$ | $3.24^{cd}$ | $0.050^{b}$ |
| γ150Gy | $2.70^{abcdef}$ | $4.41^{d}$ | $1.27^{de}$ | $4.83^{bcd}$ | $0.002^{b}$ |
| SA2H | $4.30^{abcdef}$ | $23.94^{bcd}$ | $10.48^{abcde}$ | $14.60^{abcd}$ | $0.062^{b}$ |
| SA6H | $0.50^{def}$ | $5.72^{d}$ | $0.00^{e}$ | $0.00^{d}$ | $0.000^{b}$ |
| γ100Gy + SA2H | $1.70^{abcdef}$ | $45.40^{abcd}$ | $11.32^{abcde}$ | $11.00^{abcd}$ | $0.047^{b}$ |
| γ100Gy + SA6H | $0.40^{ef}$ | $12.95^{d}$ | $6.47^{cde}$ | $4.28^{bcd}$ | $0.029^{b}$ |
| γ150Gy + SA2H | $0.60^{def}$ | $6.38^{d}$ | $0.18^{e}$ | $3.00^{cd}$ | $0.001^{b}$ |
| γ150Gy + SA6H | $1.80^{abcdef}$ | $35.70^{abcd}$ | $9.87^{abcde}$ | $8.93^{abcd}$ | $0.043^{b}$ |

**Note:** * Means followed by the same letters in a column are not significantly different ($p \leq 0.05$); * Means followed by the different letters in a column are significantly different ($p \leq 0.05$).

*Source: Herwibawa et al. (2019)*

chromosomes at meiosis. Seeds of Rojolele were induced by mutagenic agents. Gamma ($\gamma$) irradiation is a popular physical mutagen because of the shorter wave length and possess strong energy per photon that can penetrate deeply into the plant tissue compared to the x-rays. Thus, $\gamma$ irradiation is commonly use to improve agronomic, physiological, and grain yield of many crops by irradiating the seeds with suitable doses (El-Degwy, 2013). Development of optimum mutation technique is expected to provide valuable information in the improvement agronomical and grain yield characters of Rojolele rice variety.

Due to Rojolele is sensitive to drought stress condition even in the low level of drought -0.03 MPa, Herwibawa *et al.* (2019) developed effective mutation techniques to improve drought resistance level of Rojolele. The mutation techniques used two different kinds of mutagen, gamma ($\gamma$) irradiation with different doses (100Gy and 150Gy) and sodium azide (SA) along with variety soaking time (2 and 6 hours), and also combination of $\gamma$ irradiation and sodium azide (Tables 4 and 5). Several studies reported that the induction mutation techniques with $\gamma$ irradiation, sodium azide, and their combination increase the grain yield up to 7-40% (Shehzad *et al.*, 2011; Siddiqui and Singh, 2010; Dwiningsih *et al.*, 2020) and can increase the drought resistance in the level -0.0021 to -0.0077 MPa (Aurabi *et al.*, 2012; He *et al.*, 2009; Adil *et al.*, 2022). Based on the vegetative and reproductive performance of mutated Rojolele under drought, the best techniques to mutate Rojolele can be determined and used to develop drought resistance Rojolele rice cultivar. The results showed that the M1 generation of Rojolele have diverse vegetative and reproductive performances under drought stress condition based on their mutagen. Vegetative performance, including plant height, root length, leaf area, culm diameter, and number of tillers among the mutation techniques showed no significantly different (Table 4). Furthermore, based on the grain yield traits such as number of seeds per panicle, filled seed percentage, 1000 seeds grain weight, and harvest index, mutation technique with mutagen $\gamma$100Gy+ SA2H showed the most drought resistance performance of mutated Rojolele (Table 5). This mutation technique can be used to develop drought resistance Rojolele rice variety.

Since Rojolele has high plant stature that susceptible to lodge and long duration for the maturity, more than 4 months that make the productivity of Rojolele become low. In order to overcome those problems, Center for Isotope and Radiation Application, National Nuclear Energy Agency (CIRA-NNEA) developed semi-dwarf and early maturity in 3.5 months of Rojolele by ionized-seed irradiation at a dose of 200 Gy (Dewi *et al.*, 2020). CIRA-NNEA is the only institute that is conducted in mutation breeding of rice in Indonesia since 1960 and has been released about 32 mutated rice varieties, including Rojolele.

## 7. Transgenic Lines of Rojolele

Rojolele is susceptible to several diseases, such as yellow stem borer (*Scirpophaga incertulas*). The larvae of *S. incertulas* attack rice plants at any developmental stage by eating the rice stem. At the vegetative stage, the larva

**Table 6: Agronomic Characteristics of Wild Type and Six Transgenic Lines of Rojolele**

| Agronomic Characteristics | Rojolele | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Wild Type** | **P8** | **Q20** | **U10** | **W3** | **X22** | **Y7** |
| Plant height (cm) | 175.18$^{a}$ | 143.62$^{bc}$ | 146.14$^{bc}$ | 150.2$^{bc}$ | 157$^{b}$ | 144.68$^{bc}$ | 141.56$^{c}$ |
| Leaf length (cm) | 73.19$^{a}$ | 69.23$^{bc}$ | 67.91$^{c}$ | 71.82$^{ab}$ | 73.93$^{a}$ | 67.00$^{c}$ | 68.45$^{c}$ |
| Leaf width (cm) | 1.87$^{b}$ | 2.02$^{a}$ | 2.01$^{a}$ | 1.88$^{b}$ | 1.96$^{ab}$ | 1.98$^{ab}$ | 2.03$^{a}$ |
| Diameter of stem (cm) | 2.80$^{a}$ | 2.58$^{ab}$ | 2.65$^{ab}$ | 1.64$^{ab}$ | 2.67$^{ab}$ | 2.29$^{c}$ | 2.55$^{b}$ |
| Number of tillers | 19$^{a}$ | 16$^{ab}$ | 16$^{ab}$ | 13$^{b}$ | 18$^{a}$ | 18$^{a}$ | 13$^{b}$ |
| Number of productive tillers | 15$^{a}$ | 10$^{bc}$ | 13$^{ab}$ | 8$^{c}$ | 10$^{bc}$ | 9$^{c}$ | 10$^{bc}$ |
| Flowering time (das) | 116$^{c}$ | 120$^{b}$ | 122$^{b}$ | 127$^{a}$ | 117$^{c}$ | 117$^{c}$ | 122$^{b}$ |
| Grain maturity time (das) | 154$^{e}$ | 160$^{cd}$ | 161$^{bc}$ | 170$^{a}$ | 158$^{d}$ | 157$^{d}$ | 163$^{b}$ |

**Note:** * Means followed by the same letters in a column are not significantly different ($p \leq 0.05$); * Means followed by the different letters in a column are significantly different ($p \leq 0.05$).

*Source: Chairunisa andNugroho (2020)*

**Table 7: Grain Yield Components Of Wild Type And Six Transgenic Lines of Rojolele**

| Grain Yield Components | Rojolele | | | | | | |
|---|---|---|---|---|---|---|---|
| | Wild Type | P8 | Q20 | U10 | W3 | X22 | Y7 |
| Panicle numbers | 15[a] | 10[bc] | 13[ab] | 8[c] | 10[bc] | 9[c] | 10[bc] |
| Panicle length (cm) | 36.07[a] | 31.33[cd] | 30.35[d] | 31.47[cd] | 32.36[bc] | 33.43[b] | 30.58[d] |
| Filled grain numbers | 132[a] | 83[b] | 84[b] | 81[b] | 82[b] | 88[b] | 81[b] |
| Unfilled grain numbers | 44[c] | 53b[c] | 41[c] | 49[bc] | 78[a] | 67[ab] | 46[c] |
| Total grain number | 173[a] | 136[c] | 125[c] | 130[c] | 160[ab] | 155[b] | 127[c] |
| Weight of 1000 grains (gr) | 30.49[a] | 27.22[c] | 28.94[abc] | 30.60[a] | 28.24[bc] | 28.95[abc] | 29.6[ab] |

**Note:** * Means followed by the same letters in a column are not significantly different ($p \leq 0.05$); * Means followed by the different letters in a column are significantly different ($p \leq 0.05$).

*Source: Chairunisa and Nugroho (2020)*

attacks the plant's growth point at the tip of the rice plants that cause crop failure is called deadheart. At the reproductive stage, the larva eats the rice panicle and cause significant grain yield loss is called whitehead. Development of Rojolele rice lines which resistant to yellow stem borer cannot be developed by conventional rice crossing because of the resistant rice parental is not available. Resistant line of Rojolele can be developed by genetic transformation method by inserting transgene Cry1B::Cry1Aa from *Bacillus thuringiensis* (Bt) into rice plant genome. By inserting Cry1B::Cry1Aa, transgenic line of Rojolele can produce crystal protein (cry) that have insecticidal action against *S. incertulas* (Hofte and Whiteley, 1989). Transgenic line of Rojolele which resistant to yellow stem borer has developed by the Research Center for Biotechnology in Indonesia and developing six T7 generation with stable inheritance (Chairunisa and Nugroho, 2020). These six lines were P8, Q20, U10, W3, X22, and Y7. Moreover, Nugroho *et al.* (2020)described that the transgene is stable inheritance until T10 generation of the six transgenic lines. All of the transgenic lines showed less in agronomic

**Table 8: Resistant Scores of Wild Type and Six Transgenic Lines of Rojolele**

| Rojolele | Observation Time (Day After Infection) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | | | | 7 | | | | 10 | | | | 14 | | | | 21 | | | |
| | PD (%) | D | S | C | PD (%) | D | S | C | PD (%) | D | S | C | PD (%) | D | S | C | PD (%) | D | S | C |
| Wild type | 100 | 116.7 | 9 | HS | 85.7 | 100 | 9 | HS | 85.7 | 97.9 | 9 | HS | 85.7 | 85.7 | 9 | HS | 85.7 | 85.7 | 9 | HS |
| P8 | 14.3 | 16.7 | 3 | MR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR |
| Q20 | 42.9 | 50 | 7 | S | 14.3 | 16.7 | 3 | MR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR |
| U10 | 28.6 | 33.3 | 7 | S | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR |
| W3 | 28.6 | 33.3 | 7 | S | 28.6 | 33.3 | 7 | S | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR |
| X22 | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR |
| Y7 | 14.3 | 16.7 | 3 | MR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR | 0 | 0 | 0 | HR |

**Note:** * Means followed by the same letters in a column are not significantly different ($p \leq 0.05$); * Means followed by the different letters in a column are significantly different ($p \leq 0.05$).

*Source: Chairunisa and Nugroho (2020)*

characteristics and grain yield components compared to Rojolele wild type (Tables 6 and 7). This is because the transgene Cry1B::Cry1 might be inserted in a region where the growth regulation present which affects the plant development. The transgenic line which have similarity with Rojolele wild type is W3.

Yellow stem borer attacks the Rojolele rice plants in both vegetative and generative stages. Rice plants experiencing deadheart might recover but could decrease the grain yield up to 30%. Meanwhile, when rice experiencing whitehead is not able to recover and consequently total yield loss (Wunn *et al.*, 1996; Alshiekheid *et al.*, 2022; Alkahtani *et al.*, 2022). Based on the resistant scores, all of the six transgenic lines could be classified as highly resistant to the yellow stem borer, while the wild type of Rojolele belongs to highly susceptible (Table 8). The resistance performance of the transgenic lines increased by the time. At the early stages of infection, six transgenic lines showed variation in resistance performance could be because of the difference gene expression level of Cry1B::cry1Aa.

Transformation efficiency of transgene γ-glucuronidase (gus) and hygromycin resistance (hpt) into Rojolele rice variety by using *Agrobacterium tumefaciens* strain EHA101 (pAFT14) was increased up to 23% which similar to the reference rice variety Nipponbare (Rachmawati *et al.*, 2004). This efficiency due to the modification of C composition and pH in the growth medium in order to support proliferation of the callus which showed a compact and nodular appearance. Rojolele showed the highest transformation efficiency compared to the others Javanica rice cultivars, such as Menthik, Bulu, Tenggulang, and Situpatenggang (Rachmawati and Anzai, 2006; Dwiningsih and Alkahtani, 2022). Then, scutellum-derived calli were co-cultivated with *A. tumefaciens* strains EHA101 which carried plasmid pAFT14 contained both the gus and hpt genes. In the T0 transgenic lines, the copy number of the transgene gus and hpt were varied (one to three). These transgenes were inherited and expressed in the progeny lines.

Lactoferrin gene have been introduced to Rojolele by Agrobacterium-mediated transformation (Rachmawati *et al.*, 2014). Lactoferrin plays an important role as a nutraceutical food due its function against virus and microbial infection. The expression of human lactoferrin (hLF) in rice seeds under the control of ubiquitin-1 promoter. This lactoferrin was genetically introduced to the peptide of the rice glutelin. In the mature seeds, the expression of hLF gene increased significantly, but not in the vegetative tissues. The expression level of hLF varied among the transgenic lines. Based on the Enzyme-Linked ImmunoSorbent Assay (ELISA) analysis, transgenic lines TR-7, TR-8, and TR-10 which contained 2, 1, and 3 copies of hLF gene showed high level expression of hLF gene. According to Ma *et al.* (2003) copy number of transgene influenced the expression level of the transgene. The expression of hLF gene in the Rojolele transgenic seeds was stable up to three consecutive generations. Generally, hLF gene expression improved during grain-filling stage. The insertion of hLF gene to the Rojolele rice genome did not change the chromosome number and only change the chromosome size. Both wild type and transgenic lines of Rojolele displayed metacentric chromosome which indicated that Rojolele have not been crossing with others rice varieties in rice breeding program (Singh, 1999).

## 8. Future Perspectives for Rojolele Breeding

Rojolele rice cultivation is facing important challenges from drought, diseases, long duration, and lodging. Thus, breeding Rojolele cultivars with resistance to drought, diseases, lodging, and short duration is the major focus for Rojolele rice improvement. Since Rojolele have not been crossing with others potential rice varieties in rice breeding program, mapping population with Rojolele as one of the parent need to be created to identify genomic regions which responsible to the important traits, including F1, F2, backcross, Near-Isogenic Line (NIL), and Recombinant Inbred Line (RIL) populations. These populations are important in molecular breeding program. Recently, molecular breeding program, including Quantitative Trait Loci (QTL), Genome-Wide Association Study (GWAS), genomic selection, and genome editing can be applied to improve Rojolele characteristics.

Advanced technology in genomic sequencing supports the acceleration of molecular breeding in Rojolele rice cultivar. In order to identify and clone functional genes which control the life duration and resistance to drought, diseases, and lodging; molecular, biochemical, and physiological mechanisms related to these traits need to be analyzed. The whole genome sequencing of Rojolele has to be done which will help in deeper understanding the molecular basis of the complex traits in Rojolele, and open up new perspective areas of research in Rojolele, thereby providing new dimension to Rojolele rice breeding. Many novel genes associated with important traits need to be discovered by using Rojolele genetic background. In order to advance Rojolele rice cultivation, molecular breeding program need to be applied to develop Rojolele with desired characteristics. Pyramiding genes regulating short life duration and resistance to drought, diseases, and also lodging, while retaining the premium grain characteristics, fragrance aroma, and cooking quality of Rojolele rice will go a long way in overcoming the problem of water use efficiency, pesticide residue, decreasing the cost of rice cultivation, and increasing profit of the farmers. The success of molecular breeding in Rojolele is dependent on rice consumer acceptance.

## 9. Conclusion

Several agricultural researchers have studied Rojolele in terms of its morphological characteristics, grain quality properties, and cultivation techniques but not in the genomic characteristics and molecular breeding. Despite susceptible to drought, yellow stem borer, and lodge, Indonesian farmer have learned to successfully cultivate Rojolele by applying practical skills, leading to increase the rice productivity. Improving the resistance to drought, yellow stem borer, and lodge is the major focus for Rojolele rice improvement. Rice consumers demand high quality of rice grains like Rojolele characteristics which leading increased the value of Rojolele. Development of Rojolele rice cultivar with high grain yield, combined with low rice cultivation cost, and provide high profit to the farmers, is an important breeding objective of Rojolele which are widely-accepted by the farmers. It is crucial to understand the genetic basis of Rojolele agronomic and physiology characteristics, followed by extensive application of advanced genomic technology and it will improve the economics of Rojolele rice cultivation.

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