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Analysis of Genetic Diversity and Relationships of *Ipomoea batatas* L. based on Morphological Characters after Induction of the Elicitors Methyl Jasmonate and Salicylic Acid

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ABSTRACT

Background: Genetic diversity also determines a population's success in adapting to changing environments. Genetic diversity and kinship relationships can be analysed through morphological character markers, which help avoid duplication and germplasm collection. To optimise the genetic expression of a plant, including morphological characters, methyl jasmonate and salicylic acid elicitors can be used to induce the process.

Methods: The objectives of this research are 1) to describe the morphological characteristics of sweet potato varieties cultivated by farmers in Tomohon City, 2) to analyse the genetic diversity and kinship relationships of sweet potato plants based on morphological characters after induction of the elicitors methyl jasmonate and salicylic acid. Research stages: 1) Sweet potato collection, 2) Planting sweet potato tubers and cuttings, 3) Making beds and fertilising Petroganik, 4) Application of elicitor methyl jasmonate 60 ppm and salicylic acid 0.45 g/L carried out at 15 days after planting (DAP) and 30 DAP and observed morphological characters at 50 DAP. Qualitative and quantitative morphological data were analysed for kinship relationships using SPSS version 25. The stages of cluster analysis are: 1) Scoring qualitative morphological data, 2) Determining the minimum and maximum number of clusters, 3) Cluster analysis using the intergroup relationship method produces a dendogram, 4) Interpreting the analysis results.

Result: The results of research in Tomohon City obtained seven varieties of sweet potato, namely the Local purple variety (UJ1), Local white variety (UJ2), Cilembu variety (UJ3), Antin 1 variety (UJ4), Sarwentar variety (UJ5), Dungkul variety (UJ6) and Sari variety (UJ7). The results of kinship analysis based on qualitative morphological characters in the form of a dendrogram formed two main groups. Two clusters were formed, namely cluster 1 consisting of UJ1, UJ2 and UJ3 and cluster 2 consisting of UJ4, UJ5, UJ6 and UJ7. Two clusters of quantitative morphological characters were formed: cluster 1, comprising UJ1, UJ2, UJ3, UJ4 and UJ6 and cluster 2 comprising UJ5 and UJ7.

Key words: Elicitors, *Ipomoea batatas,* Morphological characters.

INTRODUCTION

The diversity of sweet potatoes (*Ipomoea batatas*) in Indonesia is very high; therefore, complete identification, description and characterisation are necessary to conserve germplasm and develop resistant varieties. Germplasm conservation is essential to prevent genetic erosion and preserve plant genetic resources. Searching for plant varieties through genetic diversity analysis can be conducted using morphological character markers (Musyarifah *et al*., 2018; Ramadhanti and Waluyo, 2021; Qiao *et al*., 2022).

Genetic diversity also plays a crucial role in determining a population's ability to adapt to a changing environment. The advantage of morphological character markers is that they are cheap and easy to apply. The observed diversity is phenotypic diversity resulting from genotypic differences. Character and genotypic diversity help identify patterns of genotypic grouping in specific populations based on observed characters and can serve as a basis for selection activities (Agustina and Waluyo, 2017; Hetharie *et al*., 2018).

Morphological characters for analysing genetic diversity and kinship relationships help avoid duplication and germplasm collection (Safitri *et al*., 2017; Avivi *et al*., 2019; Guseva *et al*., 2022). Apart from that, characterisation is used to identify essential traits of plants, such as resistance, anatomy and agronomic properties. Several researchers

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have conducted morphological characterisation studies to analyse genetic diversity and kinship relationships in plants, including Bogor beans (Sania *et al*., 2021) and sweet potatoes (Lestari *et al*., 2020; Damayanti *et al*., 2021; Wicaksono *et al*., 2022).

To optimise the genetic expression of a plant, including its morphological characteristics, it must be induced using an elicitor. The mechanism of elicitor action involves causing electron flow, Ca^{2+} influx, cytoplasmic acidification, ROS production, NADPH oxidation activation and activation of protein kinase phosphorylation. This activates the signalling pathways of salicylic acid, jasmonic acid and methyl jasmonate. These signals activate transcription factors and gene expression of secondary metabolites (Mendoza *et al*., 2018). Jasmonic acid can induce hormone synthesis and gene expression. Jasmonic acid mediates plant responses to biotic and abiotic stresses through interactions with ethylene and abscisic acid (Chini *et al*., 2016; Huang *et al*., 2017). Jasmonic acid is a natural compound in plants as a resistance response to pathogen attacks and external damage (Gomi, 2020; Geupil *et al*., 2020; Torres *et al*., 2020).

As an endogenous hormone, salicylic acid plays a role in various plant physiological processes, including protein synthesis and response to abiotic stress (Khatun *et al.,* 2016). Salicylic acid works synergistically or antagonistically with other phytohormones (Tahani, 2016). Salicylic acid is a chemical compound and phytohormone that stimulates cell reproduction and plant tissue growth and increases plant systemic resistance (SAR).

The application of methyl jasmonate to plants provides various responses, such as inducing morphoanatomical properties in *Helianthus annus*, *Solanum lycopersicum* and *Glycine max* (Li *et al*., 2018), increasing germination and photosystem II photosynthesis in *Brassica oleracea* (Sirhindi *et al*., 2020) and resistance with accumulation of secondary metabolites (Tam-Ho *et al*., 2020). In plants, salicylic acid acts as a phytohormone (Arif *et al*., 2020; Van Butselaar and Van den Ackerveke, 2022), modulates plant nutrient uptake, is involved in the pathogenesis process (Dempsey and Klessig, 2017) and fights abiotic stress (Liu *et al*., 2022). Administration of elicitors methyl jasmonate and salicylic acid will enhance various metabolic processes, morphology and genetic expression.

Several researchers have researched sweet potatoes. Reddy *et al*., (2018) characterised the morphology of sweet potato cultivars. The results showed that tuber production per planted plant was positive, along with several vegetative characteristics. Mary *et al*. (2023) examined the effect of season and K nutrients on the distribution of photosynthate content. The results showed that potassium application of up to 75 kg K_2O/ha increased sweet potato yield, total chlorophyll content and tuber K content. Furthermore, Kabede *et al*. (2022) used Morpho-sensory Sweet Potato varieties, and the results showed differences in adaptation of eight varieties with different tuber yields.

Rampe *et al*. (2021) and Rampe *et al*. (2022) researched the induction of salicylic acid and methyl jasmonate elicitors in Cilembu sweet potato varieties. The results showed an increase in quantitative morphological and anatomical characters. Genetic analysis studies and the relationship between sweet potatoes and the induced elicitors methyl jasmonate and salicylic acid can complete the data as a reference for developing resistant varieties. The research objectives are as follows: 1) Describe the morphological characteristics of sweet potato varieties cultivated by farmers in Tomohon City. 2) Analyze the genetic diversity and relationship of sweet potato plants based on morphological characters after induction of the elicitors methyl jasmonate and salicylic acid.

MATERIALS AND METHODS

Research activities were carried out in experimental gardens located in Tomohon City, North Sulawesi Province and The Advanced Biology Laboratory of the Biology Department at Sam Ratulangi University, Indonesia in 2023. The study used a completely randomised design, with three replications.

Research procedures

Sweet potato collection

Sweet potatoes were collected from farmers in five sub-districts of Tomohon City as cuttings and tubers.

Planting sweet potato tubers and cuttings

The collection of tubers and cuttings is planted until they sprout, serving as a source of cuttings for research activities.

Making beds and fertilising petroganik

The beds are given Petroganik organic fertiliser two weeks before planting the cuttings. Sweet potato cuttings measure 15-25 cm long, with at least two stem segments. Plant sweet potato cuttings by immersing 2/3 of the stem cuttings in the soil. Weeding of plants is carried out at 28 and 56 days after planting (DAP). For qualitative morphological observation, sweet potatoes are grown in polybags.

Elicitors methyl jasmonate and salicylic acid application

The elicitor application of 60 ppm methyl jasmonate and 0.45 g/L salicylic acid was performed at 15 and 30 DAP. The concentrations of methyl jasmonate and salicylic acid were used by research results (Rampe *et al*., 2021; Rampe *et al*., 2022).

Observation of morphological diversity

We observed morphological characters at 50 DAP. Sweet potato morphology data was described, namely, qualitative data including leaf shape, leaf curve type, leaf tip, leaf base, leaf edge, leaf flesh, young leaf colour, old leaf colour, leaf surface, stem shape, stem type, stem surface, stem growth direction, branch growth direction, soil cover, stem segments, stem segments, stem diameter, stem diameter, stem colour, stem colour, tuber shape, tuber skin colour and tuber colour (Damayanti *et al*., 2021).

Data analysis

Qualitative and quantitative morphological data were analysed for kinship relationships using SPSS version 25. The stages of cluster analysis are: 1) Scoring qualitative morphological data, 2) Determining the minimum and maximum number of clusters, 3) Cluster analysis using the intergroup relationship method produces a dendogram, 4) Interpreting the analysis results.

RESULTS AND DISCUSSION

Research activities began with a collection of sweet potato plants cultivated by farmers in Tomohon City. The results of the collection of sweet potato plants are tubers and cuttings; then, a nursery is created for seed stock. The collection of sweet potato plants obtained is shown in Fig 1.

Morphological description

Local purple variety (UJ 1)

Leaves are triangular, not incised. The tip of the leaf is pointed with a curved leaf base. The edges of the leaves are flat and the flesh of the leaves is thin and soft (FLTS). Young leaves are light green and mature to a dull green hue. The surface of the leaves is bare. The underside of the leaves is purple. Stems are round and hard. The stem surface is smooth with a horizontal growth direction. Medium stem segments with very long stem stems. Branches grow perpendicular. Small diameter stem. The colour of the stem is bright green with green stalks at the base. The growth of this type of sweet potato is spreading rapidly. The purple tubers are small and elongated.

Local white variety (UJ 2)

Leaves are lance-shaped, share. The tip of the leaf is pointed with a curved leaf base. The edges of the leaves are flat and FLTS. Young leaves are green and become dark green when mature. The leaf surface is bald; the leaf veins are purple on the lower side. The leaf stalks are purple near the leaf blade-purple leaf book. Stems are round and hard. The stem surface is smooth, with a horizontal growth direction, characterised by medium stem segments with long stems. Branches grow horizontally. Small diameter stem. The colour of the stem is bright green with green stalks at the base. The growth of this type of sweet potato is spreading rapidly.

Cilembu variety (UJ 3)

The leaf shape is finger-shaped, the leaf edges are flat, the colour of the young leaves is purplish green and the older leaves are green. The lower veins of the leaves are green; the leaf stalks are green. Stems are round and hard. The stem surface is smooth, with a horizontal growth direction, characterised by medium stem segments with long stems. Branches grow horizontally. Small diameter stem. The stem is purplish-green, with a green stalk at its base.

Antin 1 variety (UJ 4)

Leaves are lance-shaped notched. The tip of the leaf is pointed with a curved leaf base. The edges of the leaves are flat and FLTS. Young leaves are yellowish green and become dark purple-green when mature. The surface of the leaves is bare. The lower leaves are purple, the leaf stalks are purplish green and long and the nodes are purple. Stems are round and hard. The surface of the stem is smooth with a semi-erect growth direction. Short stem

Fig 1: Sweet potato (*Ipomoea batatas*).

segments with medium stem stems. Branches grow perpendicular medium stem diameter. The stem colour is green. The growth of this type of sweet potato is spreading rapidly.

Sarwentar variety (UJ 5)

Leaves are heart-shaped, not incised. The tip of the leaf is pointed and the base of the leaf is flat. FLTS and the flesh of the leaves is thin and soft. Leaves are dull green. The surface of the leaves is bare. Leaf veins are green; petioles are green; nodes are green and petioles are medium-sized. The stem is square with a complex type. The surface of the cotton stems with a perpendicular growth direction. Short stem segments with short stem stems. Branches grow perpendicular. Large diameter stem. The stem is green, with a green stalk at the base.

Dungkul variety (UJ 6)

Heart-shaped leaves. The tip of the leaf is tapered with a notched base. FLTS and the flesh of the leaves is thin and soft. Young leaves are light green, while mature leaves are dark green. The leaf surface is smooth (shiny). The leaf veins are purple, the nodes are green and the leaf stalks near the leaf blade are purple. Stems are round and hard. The stem surface is smooth, with a perpendicular growth direction and features medium stem segments with long stems. Branches grow spread out. Medium diameter stems. The colour of the stem is bright green with green stalks. The growth of this type of sweet potato is spreading rapidly.

Sari variety (UJ 7)

Leaves are lance-shaped and notched. The tip of the leaf is pointed with a curved leaf base. The edges of the leaves are flat and FLTS. Young leaves are reddish-green and mature to a purplish-green colour. The surface of the leaves is bare. Stems are square-shaped and of a complex type. The surface of the stem is smooth with a semi-erect growth direction. Medium stem segments with medium stem stems. Branches grow perpendicular. Medium diameter stems. The colour of the stem is bright green with green stalks. This type of sweet potato grows high and covers the ground.

The results of hierarchical cluster analysis, which show the relationship between sweet potato varieties based on

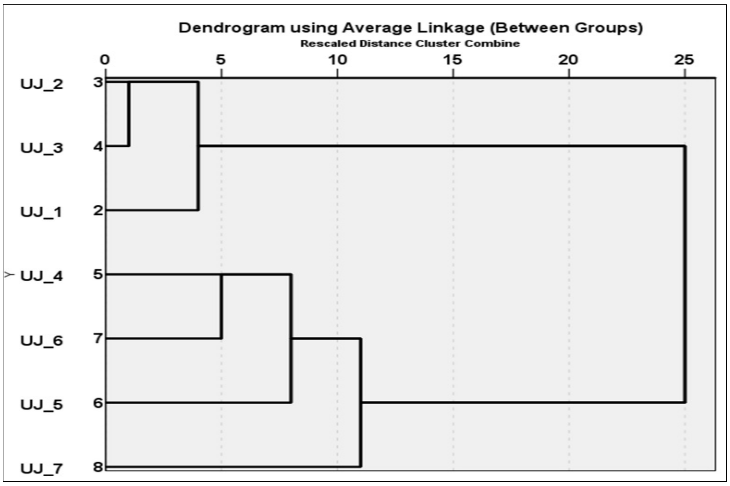

Fig 2: Dendogram of qualitative morphological characters of sweet potato.

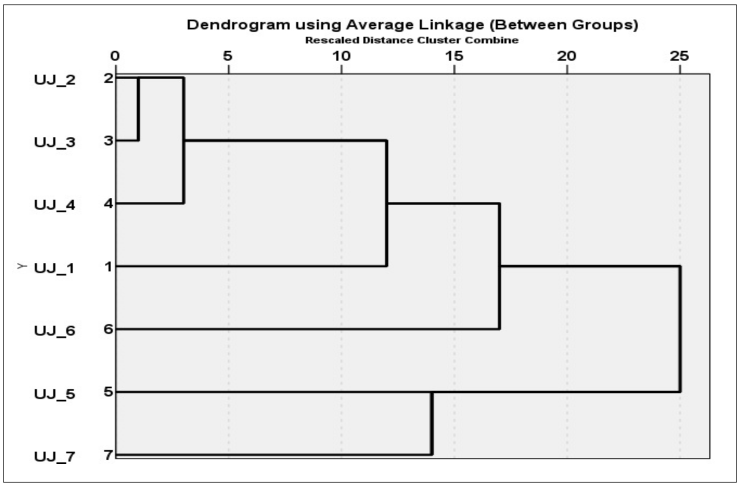

Fig 3: Dendogram of quantitative morphological characters of sweet potato.

the qualitative morphology of the collection of sweet potato plants, including tubers and cuttings, are then used to create a nursery for seed stock. The results of hierarchical cluster analysis are in Fig 2.

As a result of clustering, stage 1 formed a cluster UJ2 and UJ3 (distance 8.000), stage 2 forms klasters UJ2 and UJ1 (distance 13.000), stage 3 forms clusters UJ4 and UJ6 (distance 14.000), stage 4 forms clusters UJ4 and UJ5 (distance 18.000), stage 5 forms clusters UJ4 and UJ7 (distance 22.000) and stage 6 forms clusters UJ1 and UJ4 (distance 40.000). The smaller the euclidean distance, the closer the kinship relationship and the more clusters it forms. The two main clusters are cluster 1, consisting of UJ2, UJ3 and UJ1 and cluster 2 consisting of UJ4, UJ6, UJ5 and UJ7.

The results of quantitative morphological character observations were obtainedthe average range of lamina length (10.02-19.98 cm), lamina width (9.02-13.80 cm), petiolus length (15.88 - 22.86 cm), petiolus diameter (0.82-1.68 cm), caulis diameter (0.88-1.82 cm), number of branches was obtained. (3.33-4.67), branch length (104.82 -183.66 cm) and internode length (10.55 - 18.40 cm) (Table 1). Dendogram of quantitative morphological characters of sweet potato (Fig 3).

The results of the cluster analysis show that stage 1 forms clusters UJ2 and UJ3 (distance 10,004), stage 2 forms clusters UJ2 and UJ4 (distance 13,959), stage 3 forms clusters UJ1 and UJ2 (distance 26,081), stage 4 forms clusters UJ5 and UJ7 (distance 27,754), stage 5 forms clusters UJ1 and UJ6 (distance 32,480) and stage 6 forms clusters UJ1 and UJ5 (distance 44,016). The two main clusters are cluster 1, consisting of UJ2, UJ3, UJ4, UJ1 and UJ6 and cluster 2, consisting of UJ5 and UJ7.

Resistance induction activates plant resistance to pathogens and insects using an elicitation method with the chemical elicitors methyl jasmonate and salicylic acid. Rampe *et al*. (2021) used a single elicitor with a salicylic acid elicitor and Rampe *et al*. (2022) used the elicitor methyl jasmonate. This study used a combination of elicitors, methyl jasmonate and salicylic acid.

A prolonged, dry season occurred during the research activities, which may have resulted in suboptimal growth due to insufficient availability. Elicitor application is not optimal when water availability is a limiting factor. Salicylic acid plays a role in lignin biosynthesis, while phytoalexins protect plants from fungi, bacteria and viruses. Salicylic acid overcomes attacks by biotrophic pathogens (active in living tissue) and viruses. Exogenous salicylic acid can influence plant growth, photosynthesis, water relations and the activity of several types of enzymes that play a role in biotic and abiotic stress (Zamaninejad *et al*., 2013). Salicylic acid regulates plant responses to environmental and everyday stress conditions through cross-talk signals with other phytohormones.

Qualitative morphological character cluster analysis using the intergroup linkage cluster method formed two

Table 1: Quantitative morphological characters of sweet potato plants.

Sweet potato variety	Morphological characters							
	Long lamina (Cm)± SD	WideLamina (Cm)± SD	Number of leaves± SD	Petiolus lenght (Cm)± SD	Diameter petiolus (Cm)± SD	Diameter caulis (Cm)± SD	Number of branches±SD	Branches lenght (Cm)±SD	Internode lenght (Cm)±SD
UJ 1	10.32±1.45	9.02±0.46	23.67±0.58	18.24±0.98	0.82±0.08	0.88±0.06	3.33± 0.58	145.32±1.80	12.78±0.92
UJ 2	10.02±0.72	10.24± 1.42	24.67±1.15	18.62±1.64	0.98±0.06	0.91±0.06	3.67±0.58	149.70± 2.30	12.64±1.66
UJ 3	17.70± 1.40	13.02±0.88	27.33±2.08	18.06±2.24	1.52±0.08	1.24±0.06	4.00±1.00	118.09±2.62	10.55±1.64
UJ 4	19.98± 0.67	13.20±0.45	27.00±1.00	20.97±0.68	1.24±0.08	1.66±1.18	4.67±0.58	108.22± 6.62	16.41±1.88
UJ 5	12.4±1.22	10.28±0.98	27.33±2.08	15.88±1.78	1.68±0.08	1.82±1.34	4.00±1.00	104.82±2.24	18.40±2.02
UJ 6	13.6±1.09	12.8±1.02	24.67±1.15	17.34±0.82	1.30±0.09	1.38±1.88	3.33± 0.58	126.66±1.64	15. 66±2.28
UJ 7	13.2±0.98	13.80±0.88	27.33±2.08	22.86±0.94	1.34±1.02	1.62±0.99	3.67±0.58	183.66 ±2.88	18.02±1.16

main groups. Two clusters were formed, namely cluster 1 consisting of UJ2, UJ3 and UJ1 and cluster 2 consisting of UJ4, UJ6, UJ5 and UJ7. Group 1 was observed based on various qualitative morphological characters that showed many similarities, namely leaf morphology (leaf shape, leaf margin, leaf tip, leaf base, leaf flesh, young leaf color, mature leaf colour, leaf surface), stem (stem shape, stem surface, growth direction) and tuber shape. In Group 2, similarities in leaf morphology (leaf margin, leaf flesh, young leaf color, leaf surface, stem color), stem (stem surface, growth direction, stem color) and tuber shape were observed.

The quantitative morphological character dendrogram (Fig 3) shows the differences from the qualitative morphological character dendrogram (Fig 2). The quantitative morphological characters used were lamina length, width, number of leaves, petiole length, petiole diameter, caulis diameter, number of branches, branch length and internal node length. In Fig 3, the quantitative morphological character dendrogram shows two main clusters. Two clusters were formed: cluster 1, comprising UJ2, UJ3, UJ4, UJ1 and UJ6 and cluster 2, comprising UJ5 and UJ7. It can be observed that the morphological similarities between sweet potato varieties, as shown in the dendrogram, may differ from their secondary metabolite content.

The relationship between methyl jasmonate application and the morphoanatomy response is that salicylic acid acts as an endogenous hormone. It is known that five types of hormones play a role in plant growth and development: auxin, gibberellin, cytokinin, ethylene and abscisic acid. As a phytohormone, methyl jasmonate stimulates cell division in the meristematic area and promotes plant tissue growth while also increasing resistance through the systemic acquired resistance (SAR) system. The synthesis of hormones in plants can stimulate the division, expansion and differentiation of plant tissue, resulting in effects on quantitative morphological and anatomical characteristics. As a hormone, Jasmonic acid regulates plant adaptation to biotic stresses, including herbivore attacks and pathogen infections, as well as abiotic stresses, such as wounds, ozone and ultraviolet radiation (Huang *et al*., 2017).

The application of methyl jasmonate affects the absorption of nitrogen and phosphorus and the transport of organic compounds such as glucose (Gupta *et al*., 2017). Furthermore, salicylic acid plays a role in plant growth and development (Li *et al*., 2022). The use of these two elicitors responds to plant growth and development during the vegetative and generative phases. The formation of roots, stems and the availability of macronutrients, micronutrients and phytohormones largely determine the development of leaves. Physiological and biochemical activities of plants support genetic expression, such as morphological characteristics.

CONCLUSION

The results of measuring quantitative morphological characters obtained a range of mean lamina length (10.02 - 19.98 cm), lamina width (9.02 - 13.80 cm), petiolus length (15.88 - 22.86 cm), petiolus diameter (0.82 - 1.68 cm), caulis diameter (0.88 - 1.82 cm), number of branches (3.33- 4.67), branch length (104.82 - 183.66 cm) and internode length (10.55 - 18.40 cm). The results of kinship analysis based on qualitative morphological characters in the form of a dendrogram formed two main groups. Two clusters were formed, namely cluster 1 consisting of UJ2, UJ3 and UJ1 and cluster 2 consisting of UJ4, UJ6, UJ5 and UJ7. Two clusters of quantitative morphological characters were formed: Two clusters were formed: cluster 1, comprising UJ2, UJ3, UJ4, UJ1 and UJ6 and cluster 2, comprising UJ5 and UJ7.

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Conflict of interest

The authors declare no conflicts of interest regarding the publication of this article.

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