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Rooting response of white Potato (*Solanun tuberosum* L.) stem cuttings under three different conditions

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Abstract

The need to produce a cheap alternative and farmer level technology in the production of clean planting materials of White Potato (*Solanum tuberosum* L.) prompted the investigation on the rooting response of clones from stem cuttings of different age and number of nodes under different concentrations of synthetic plant hormone Alpha-Naphthalene Acetic Acid (ANAA). A zero generation (G_0) mother plant was used as a source of clones to examine whether it could produce roots in a sterilized medium. In a replicated split-split plot experimental design with three factors such as the age of the mother plant, number of nodes and levels of growth regulator, we found that roots emerged from clones 18 days after planting in a sterilized river sand. Significant effect on rooting was influenced by the age of cuttings (p=0.0058), number of nodes (p=0.0058) and ANAA (p=<0.0001). Moreover, significant interactions were found among age of cuttings, number of nodes and ANAA concentrations on rooting (p=0.0044). Implications for the feasibility of mass producing clean planting materials from cloning G_0 mother plant are discussed.

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Introduction

White potato (*Solanum tuberosum* L.) is a perennial crop belonging to the Solanaceae family grown mainly for its tubers (Spooner *et al.*, 2014). First cultivated 8000 years ago by the Peruvian farmers in Peru's Central Andes; now it has more than 4000 different cultivars grown globally (Niekerk *et al.*, 2016; Lutaladio *et al.*, 2009). It was initially introduced in Europe in the sixteenth century and was subsequently distributed throughout the world. Potatoes are the world's primary non-grain staple food in several countries in Europe and some parts of America due to its nutrient content; with China, India, Ukraine and Russia as top producers (Lutaladio *et al.*, 2009; Shahbandeh, 2022).

The biggest obstacle of the white potato industry in Asia, particularly Philippines is the source of clean planting materials, because potatoes are attacked by bacterial wilt disease caused by pathogen *Ralstonia solanacearum*. The conventional method of white potato propagation is through the use of tubers, but the risk is high. Other methods are the use of True Potato Seeds (TPS), and through stem cuttings (Morais *et al.*, 2018; Shiwani *et al.*, 2021). At present, the Department of Agriculture's Northern Mindanao Agri Crops and Livestock Research Complex (DA-NMACLRC) uses the tissue culture technology to mass produce seedlings and tubers as the source of potato clean planting materials, but cannot cope up with the current demand; thus there is pressure to explore other methods.

Numerous studies were conducted to explore and enhance the propagation of potato through stem cuttings. The work of Zaki and Moustafa (2018) for example, used Indole Acetic Acid (IAA) and Indole Butyric Acid (IBA) at higher concentrations reaching up to 6000 parts per million (ppm) but rooting responses of potato varieties tested differ significantly. Ezzat (2016) dipped the stem cuttings for 1 minute to various rooting hormones such as Indole-3-butyric acid Potassium salt (K-IBA) at 1000 ppm, IAA at 250, and 1-Naphthaleneacetic acid NAA at 500 mg/L. The same hormone IAA was tested by Nikmatullah *et al.* (2018), but other factors such as age of mother plant and number of nodes was included.

In this study we explored the responses and interactions at different ages of Granola white potato stem cuttings, number of nodes, and levels of hormones in terms of its rooting ability and growth. Results and its potential for tuber production are discussed.

Materials and methods

Clean and pathogen free tubers of the zero generation (G_0) mother plant from a tissue culture laboratory were obtained from NMACLRC in Dalwangan, Malaybalay City. These tubers were allowed to grow in a sterilized medium as a source of cuttings. A total of 1000 cuttings of different three ages (3-week old, 5-week old and 7-week old) were then produced from these tubers for an experiment.

The experiment followed the 3 x 3 x 3 split-split plot design with the 1st factor as the age of cuttings (factor A-main plot): 3 weeks (A_1), 5 weeks (A_2), and 7 weeks (A_3), 2nd factor as the number of nodes per cutting (Factor B - sub-plot): 2 nodes (B_1), 3 nodes (B_2) and 4 nodes (B_3); and the 3rd factor was the concentration of hormone (Factor C – sub-sub plot): negative control (C_0), 2 ppm (C_1), and 4 ppm (C_2). All treatments under each factor were replicated 3 times.

Nine different group combinations of cuttings based on age and number of nodes were prepared and hormone treatment was administered using α-Napthalene Acetic Acid (ANAA) at three different levels as factor C. Dipping time for hormone treatment was 5 minutes, and cuttings were then planted in a sterile sand medium. Care and maintenance by weeding and watering were performed for 30 days, and observations on rooting was done. Assistat version 7.7 was used for data analysis.

Results and discussion

Results revealed that potato clones from G_0 mother plant stem cuttings were able to produce roots 18 days from planting (Fig. 1) in a sterile sand medium. Factors such as age of the mother plant significantly influenced rooting [*df*=2, *F*=24.2366, *p*=0.0058], this may have been related to the different capability to concentrate soluble sugar in the stem cutting during rooting (Ahkami *et al*., 2013; Tombesi *et al*., 2015; Otiende *et al*., 2017); sugar is a pivotal factor in root formation. Auxin hormone is known to cause root growth (Teale, 2004; Husen and Pal, 2007; Overvoorde *et al*., 2010; Sosnowski *et al*., 2023), but its effects on rooting are influenced as well by the nutritional status of the stem cuttings, and that proper balance of the two is necessary for root development (Lee and Cho, 2013; Otiende *et al*., 2017).

Fig. 1. Root emergence 18 days after planting of stem cuttings in a sterilized river sand.

The number of nodes was also found to have significant effects on rooting [*df*=2, *F*=35.3463, *p*=<0.0001], with 4 nodes producing the highest number of roots (Table 2). Although there was discrepancy observed such as the lack of pattern on its effect, it is necessary that re-evaluation be done with an increased number of replications. Nevertheless, the rooting of stem cuttings as influenced by the number of nodes may have been related to nutritional deposits, most notably sugars. Moreover, nodes could have acted as sinks for auxin hormone and sugars that are transported basipetally to site for root development. Signals for root hair formation are located upstream (Lee *et al*., 2013; Zhang *et al*., 2016) and transported downstream to the site of action. This is similar to the work of Yesuf *et al.* (2021), the number of nodes exerted influence on the rooting of the Chaya plant, and these responses are similar to that of Nikmatullah *et al.* (2018). Moreover, Adugna *et al.* (2015) found out that the interaction of the number of nodes of vanilla stem cuttings and soil media significantly affects the root length, root volume, and rooting percentage of cuttings.

The concentration of growth regulator ANAA on rooting was significant [*df*=2, *F*=48.6800, *p*=<0.0001]. ANAA belongs to the same category of IAA and IBA, they are all growth regulators of the same effect and functions as the naturally occurring Auxin hormone; one of these functions is promotion of root growth (Trobec *et. al*., 2005). Auxin is responsible for root hair growth and has been shown to act via cell division (Teale *et. al*., 2004). However, at high concentration this inhibits root elongation but promotes cell differentiation and cell division at the meristem. The application of NAA at a lower concentration could downregulate the KRP2 protein, a known CDK protein inhibitor (Sanz *et al*., 2011; Menges and Murray, 2002; Teale *et al.,* 2005), and thus promote cell division.

The work of Ahmed *et al.* (2018) showed that not all varieties of white potato responded positively to hormone treatment, only two out of five varieties namely Burren and Lady Rosetta produced roots and successfully grew into an intact plant. Moreover, these two varieties responded very well at higher hormone concentrations that reached as high as 6000 ppm. This suggests variations of white potato responses to hormones. Although in this study we used alpha-Naphthalene Acetic Acid (ANAA), rooting of Granola potato variety (Fig. 1) was successful at low concentration from 1 ppm to 3 ppm and even without ANAA application (Table 2); roots emerge in 18 days from the day of planting.

Results of this study were similar to the work of Nikmatullah *et al.* (2018) in a sense that a growth regulator produced roots in white potato at 1 ppm - a very low concentration; it differs only in the kind of growth regulator that was used. The rooting of Granola without ANAA applications (Table 2) could be attributed to naturally occurring auxin hormone present at the tip of the cuttings - the tip was not removed in this study, and this hormone was transported basipetally.

Table 1. Analysis of variance of three factors: age of mother plant, number of nodes of cuttings, ANAA concentration and their interactions.

Source of variation	Degrees of freedom	Sum of square	Mean square	F-value	p-value
Blocks	2	6.75190	3.37595	25.3786 **	0.0053
Treatment a (Ta)	2	6.44807	3.22403	24.2366 **	0.0058
Error-a	4	0.53209	0.13302		
Treatment b (Tb)	2	2.66857	1.33428	35.3464 **	<.0001
Int. Ta x Tb	4	3.04400	0.76100	20.1596 **	<.0001
Error-b	12	0.45299	0.03775		
Treatment c (Tc)	2	2.86154	1.43077	48.6800 **	<.0001
Int. Ta x Tc	4	0.49338	0.12335	4.1967 **	0.0068
Int. Tb x Tc	4	13.29982	3.32495	113.1270 **	<.0001
Int.Ta x Tb x Tc	8	0.81990	0.10249	3.4870 **	0.0044
Error-c	36	1.05809	0.02939		

Ta – age of mother plant, Tb – number of nodes of cuttings, and Tc – ANAA concentration

Table 2. Mean number of roots produced as affected by age of mother plant, number of nodes and growth regulator concentrations.

Factor	Treatment	Mean number of roots
1. Age of mother plant		
	3 weeks	3.98 b
	5 weeks	3.63 b
	7 weeks	4.32 a
2. Number of nodes		
	2 nodes	3.93 b
	3 nodes	3.78 c
	4 nodes	4.22 a
3. Growth hormone concentration		
	Control (0 ppm)	4.23 a
	2 ppm	3.78 c
	4 ppm	3.92 b
A×B×C	**	
CV%-a =9.16 CV%-b =4.88 CV%-c =4.31		

Significant interactions were found among age of the mother plant, number of nodes in stem cuttings and concentration of ANAA (Table 1). This suggests that choosing a 7 week old stem cuttings from G_0 mother plant with 4 nodes even without ANAA treatment could successfully produce roots in granola variety. Positive response to rooting was also found when cuttings were treated with very low concentration of ANAA up to 4 ppm. Further testing is needed on other varieties of white potato as there may be variations in responses such as the one observed in the work of Ahmed *et al.* (2018).

Nikmatullah *et al.* (2018) argued that G_0 stem cuttings dipped in IAA could be used as planting materials for the production of potato tubers. In their work, the emergence of roots and increase in number is followed by the production of leaves and continued growth as an intact plant.

The rooting is crucial as it supports nutrition to vegetative growth up to reproduction and production of tubers. The granola variety in this study successfully produced roots and increased its numbers. Therefore, the use of stem cuttings from the G0 mother plant as a source of clean planting material is feasible and practical.

The plausibility of production of clean planting material for granola variety through cloning from stem cutting of G_0 mother plant without ANAA treatment is resolved. Further testing of this technique to other varieties however is necessary as it exhibits large scale variability in terms of rooting responses.

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