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IN VITRO SHOOT GROWTH PERFORMANCES AND RESPONSES OF POTATO (*Solanum tuberosum* L.) 'MUHZOTO' UNDER DIFFERENT TREATMENTS AND EXPLANT TYPES

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ABSTRACT. Finding optimal shoot growth performances under different treatments and revealing different growth responses of different explant types as main objectives were assessed in the research. Different treatments of 5 000; 8 000 and 11 000 lx in light intensities; 0, 25, 50, 75 and 100 ml l^{-1} in coconut water (CW) concentrations; culture media (CM) of Murashige and Skoog (MS) medium supplemented with 1.5 vitamin strength in the medium in combination with 100 mg l^{-1} myoinositol, 1 mg l^{-1} calcium pantothenate (CaP), and 0.1 mg l^{-1} gibberellic acid-3 (GA_3) (CM-1); 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP dan 0.1 mg l^{-1} GA3 (CM-2); 1 mg l^{-1} CaP and 100 ml l^{-1} CW (CM-3); and MS medium supplemented with 1.5 total vitamin strength in the medium (CM-4 as control) and shoot tip, first, second, third, fourth and fifth nodes as explant types were gradually tested in the research. Virus-free *Solanum tuberosum* L. 'Muhzoto' explants and MS medium containing 1.5 strength of vitamin were used as explant source and basic medium. Four experiments were arranged in a completely randomized design (CRD) with 6–9 replications. Maximal shoot growth performances indicated by shoot height, stem diameter, internode length, greener leaves per shoot, leaf length and width were established in explants incubated under 11 000 lx light intensity applied continuously. Adding different concentrations of CW could not improve the growth of shoots, but they induced high contamination. Though MS medium containing 1.5 vitamin strength with 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP and 0.1 mg l^{-1} GA_3 slightly improved shoot growth, there was no significant difference compared to control. Exploring shoot growth responses derived from different types of explants revealed that the shoot tips, 1st and 2nd nodes regenerated high branched shoots with the higher length of internodus; while 3rd, 4th and 5th nodes stimulated low branched shoots with higher stem diameter and the number of leaves per shoot. The branched shoots were a serious problem in preparing high-quality regenerants for 'Muhzoto' explants and significantly overcome by choosing, selecting and applying the right time on subculturing of the 'Muhzoto' explants.

Introduction

Potato (*Solanum tuberosum L.*) is one of the most important dicotyledonous tuber crops worldwide and the position is in ranking fourth in food crop after wheat, rice and maize (Husain *et al.*, 2017). In 2017, total production of potato reached 388 191 000 tons; 19 302 600 ha total harvested area; China, India and Russian Federation as the most important producing countries with an annual production of 99 205 600; 48 605 000 and 29 590 000 tons, respectively (FAOSTAT, 2019; Potatopro, 2019; Thedailyrecords,

2019). In Indonesia, the potato is also the most important vegetable commodity in the third position after hot and chilli pepper (Badan Pusat Statistik, 2019a,b). In 2019, total cultivated areas were 68 683 ha and East Java, Central Java and West Java as the main important producing provinces with an annual production of 1 284 760 tons and productivity of 18.7 tons per ha (Badan Pusat Statistik, 2019a,b,c). The fresh potato products in tuber form are sold from 12 000.00 to 20 000.00 IDR (0.69–1.15 EUR) per kg depending on their types and qualities (Pdppjkotabogor, 2020; Infopangan, 2020; Priangan, 2020; Siskaperbapo, 2020). Though the potato has high economical values, the development and production of high-quality tuber in Indonesia are constrained by the availability of good and qualified planting materials for sustainable production.

Conventionally potato can be propagated generatively using true botanical seeds and vegetatively using tubers, segments of tubers (Hidayat, 2011; Ebad *et al.*, 2015; Mehmood *et al.*, 2016; Husain *et al.*, 2017; Muñoz *et al.*, 2019) and mini-cuttings (Karjadi, 2017; Kasutjianingati *et al.*, 2018). Because potato is highly heterozygous and easily segregates on sexual reproduction, application of the true botanical seeds for commercial purposes is rarely utilized (Ebad *et al.*, 2015). Furthermore, utilizing tuber is the most common asexual propagating technique applied by potato farmers and growers involving in Indonesia. Though it may be frequently attacked by different pathogens and this method is also time-consuming and laborious, the method is the most frequently applied by Indonesian farmers. While mini-cuttings derived from terminal shoots and the single node was now generally used by Indonesian farmers to get planting materials in large quantities with lower prices than tubers (Karjadi, 2017; Kasutjianingati *et al.*, 2018). Furthermore, to reduce immersing low-quality tubers and cuttings due to gradual utilizing them continually, preparing good quality planting materials derived from *in vitro* plantlets significantly addressed.

Preparing good quality planting material for *in vivo* stage was generally initiated by producing high quality *in vitro* materials. Several *in vitro* propagation studies on the potato to establish the purpose were previously reported. The highest number of shoots/explant was obtained for 'Almera' explants cultured on MS medium supplemented with 3.0 mg l^{-1} thidiazuron (TDZ) in combination with 0.1 mg l^{-1} naphthalene acetic acid (NAA) (Khadiga *et al.*, 2009). In the 'Esprit' and 'Meridian' potato variety, maximum numbers of healthy shoots derived from shoot apex and nodal explants with well-expanded leaves per explants were produced on MS medium with 1.0 mg l^{-1} benzylaminopurine (BAP) + 1.0 mg l^{-1} GA3 (Bhuiyan, 2013). Multi shots and roots from nodal explants were established on MS medium supplemented with IBA 1.0 mg l^{-1} + NAA 1.0 mg l^{-1} + kinetin 2.0 mg l^{-1} and 2.0 mg l^{-1} IBA + 2.0 mg l^{-1} kinetin + 2.0 mg l^{-1} NAA + 1.0 mg l^{-1} 2,4-D (Gami *et al.*, 2013). MS medium containing 4.19 μM D-calcium pantothenate, 0.05 μM NAA, 0.29 μM GA_3, 30 g l^{-1} sulphur less sugar and 2 g l^{-1} gelrite stimulated a high number of leaves per shoot, internodal length, number of roots, root length, fresh and dry weight of varied tetraploid potato varieties (Venkatasalam *et al.*, 2013). MS medium with vitamins and solidified by agar without exogenous plant growth regulators and nodal cuttings were successfully produced high shootlet length, number of leaves per shootlet, number of vigorous roots and fresh mass of 'Lady Rosetta' variety (Ebad *et al.*, 2015). Maximum shoot regeneration derived from nodal explants of 'Diamont', '1533' and 'Kufri Badshah' potato varieties were determined on MS medium supplemented with 1 mg l^{-1} BAP (Kaur *et al.*, 2015). MS medium containing 0.12 mg l^{-1} of GA3 and nodal explant for 'Cardinal' variety produced maximum plant height, the higher number of nodes, reduced number of days to root initiation and took less number of days to the transferable height of the plant (Mehmood *et al.*, 2016). From those study they were informed that optimal shoot growth performances were generally established by optimation of MS medium in combination with applying concentration and combination of plant growth regulator, different varieties, solidifying agents, water; however studying and finding optimal shoot growth performances derived from different light intensities, coconut water concentrations, varied-MS media and paying more attention to growth response of different types of explant in *in vitro* culture of Indonesian potato variety, *i.e. S. tuberosum* 'Muhzoto' were not published yet.

The research was aimed to study the effect of different light intensities, coconut water concentrations, and modified-MS media on shoot growth performances of *S. tuberosum* 'Muhzoto' explants and to explore growth responses of different explant types of the variety. From the study, it was expected that optimal light intensity, CW concentration and modified-MS medium for maximal shoot growth performances and different responses of different explant types of *S. tuberosum* 'Muhzoto' were successfully established and explored. Important and unique findings of the research expected could give benefits to others.

Material and methods

Materials and explant preparation

Materials used in the study were plantlets of *S. tuberosum* 'Muhzoto' variety derived from meristem culture and virus free produced by Kalimandi Main Institute for Horticulture Seeds (KMIHS), Banjarnegara District, Central Java province, Indonesia. The plantlets were cultured in jam bottles (7 × 11.6 cm; diameter and height of bottle) containing full strength MS medium hormone-free. The plantlets generally had 5.0 cm in height with 5–6 six leaves after 30 days of culture. Each bottle contained 8–10 plantlets. The cultures were incubated on culture racks under 16 h photoperiod light incubation of cool fluorescent lamps with 5 000 lx and 24 ± 1°C just for ± a week before used as explant sources for the experiments.

Explants used in the study were shoot tip and nodus derived from KMIHS plantlets as explant source.

Explants were prepared by slicing shoot tip with a young leaf, first, second, third, fourth and fifth nodes. Explants were then cultured on a medium or different media based on treatments studied. All experiments used Murashige and Skoog (MS, 1962) medium containing 1.5 vitamin strength as a basic medium.

Shoot growth performances of 'Muhzoto' explants under different light intensities

Explants used in the study were shoot tips and nodes as described previously. While light intensities tested in the study were (1) 5 000 lx derived from 24-watt Tornado Phillip. The lamps were set in 90 cm distance from one lamp to another with 40 cm height position of lamps to rack surface; (2) 8 000 lx derived from 12-watt LED Phillip. The lamps were set in 50 cm distance from one lamp to another with 40 cm height position of lamps to rack surface; and (3) 11 000 lx derived from 19-watt LED Phillip with a similar setting as the previous treatment (no. 2). The experiment was arranged in a completely randomized design (CRD) with 9 replications. Each treatment consisted of 3 bottles. Each bottle contained 10 explants. The continuous light incubation applied in the experiment was due to the incubation as optimal condition established derived from preliminary studies and also generally applied in Indonesian potato tissue culture laboratories.

Shoot growth performances of 'Muhzoto' explants under different concentration of coconut water (CW)

Explants used in the study were shoot tips and nodes. While CW concentrations examined in the study were (1) 0, (2) 25, (3) 50, (4) 75 and (5) 100 ml l^{-1}. Application of different concentration of CW in the media was only carried out by sterilization and no filtration applied for CW. The experiment was arranged in CRD with 5 replications. Each treatment consisted of 3 bottles. Each bottle contained 10 explants. All cultures of explants were incubated in 11 000 lx light intensity derived from 19-watt LED Phillip under continuous incubation as an optimal treatment to support the optimal growth response of shoots.

Shoot growth performances of 'Muhzoto' explants under different culture media

Explants used in the study were shoot tips and nodes. Culture media (CM) of MS medium supplemented with 1.5 vitamin strength in the medium in combination with 100 mg l^{-1} myoinositol, 1 mg l^{-1} CaP, and 0.1 mg l^{-1} GA_3 (CM-1); 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP dan 0.1 mg l^{-1} GA_3 (CM-2); 1 mg l^{-1} CaP and 100 ml l^{-1} CW (CM-3); and MS medium supplemented with 1.5 total vitamin strength in the medium (CM-4 as control). The experiment was arranged in CRD with 6 replications. Each treatment consisted of 3 bottles. Each bottle contained 10 explants. All cultures of explants were incubated as described in the previous experiment.

Shoot growth response study of 'Muhzoto' explants

Shoot growth response was studied by clustering explants in each group separately. That was mean that the shoot tip was clustered with the shoot tip, the first node with the first node till the fifth node. The different clusters of explants were then used as a treatment in the study *i.e.* (1) shoot tips, (2) 1st nodes, (3) 2nd nodes, (4) 3rd nodes, (5) 4th nodes and (6) 5th nodes. The experiment was arranged in CRD with 4 replications. Each treatment consisted of 3 bottles. Each bottle contained 10 explants. All cultures of explants were incubated as described in the previous experiment.

Variables of experiments

Variables observed in all experiments carried out were (1) height of shoot (cm), (2) stem diameter (mm), (3) internodus length (cm), (4) number of leaves per shoot, (5) leaf length (mm), (6) leaf width (mm), (7) percentage of explant contamination (%), calculated by counting the number of contaminated explants divided by total explant cultured time by 100%, (8) the percentage of air roots (%), calculated by counting the number of nodes with air roots divided by total nodes time by 100% (9) number of air roots per shoot, (10) air root length (cm), (11) leaf length-width ratio, (12) percentage of branched-shoots (%), calculated by counting the number of branched-shoots divided by total shoots cultured time by 100%. The periodical observation was carried out to know the response of explant growth during each experiment conducted. All variables were observed and measured ± 30 days after culture initiation.

Statistical analysis

Data regenerated from all variables observed in each experiment were analyzed by analysis of variance (Anova) using SAS 9.1 program (SAS Institute, Cary, NC). Significant differences between means were assessed by Tukey test, P = 0.05.

Results and discussion

Shoot growth performances of 'Muhzoto' explants under different light intensities

Results of the study reveal that axillary shoots derived from nodus explants were observed 2–3 days after culture. The axillar shoots grew continually to produce new leaf primordia 4–5 days after culture. In further growth, the shoots had the height of shoots from 5.0–7.2 cm with 0.75–1.35 mm in diameter; 0.2–1.4 cm in length of internodus; 5–11 leaves per shoot; 1.0–5.0 mm leaf length and 0.5–3.1 mm leaf width (Fig. 1A). Best performances of shoot growth were exhibited on potato explants incubated under high light intensity.

Three different light intensities tested in the first experiment resulted in different responses and growth performances of potato explants. It was revealed that higher light intensity induced better growth of shoots. The potato explants cultured under 11 000 lx continually induced the best growth performances of plantlets with 6.6 cm in shoot height, 1.25 mm in stem diameter, 0.71 cm in internodus length, 9.4 number of leaves per shoot, 4.4 mm in leaf length and 2.9 mm in leaf width (Table 1; Fig. 1A). The second best treatment was determined on the potato explants incubated under 8 000 lx light intensity. While low light intensity generally stimulated non-optimal shoot growth of explants with thinner stem diameters, pale to light green performances and small leaves.

Table 1. Growth performances of 'Muhzoto' explants cultured under different light intensities

Light intensity, lx	Height of shoots, cm	Stem diameter, mm	Internodus length, cm	Number of leaves per shoot	Length of leaves, mm	Width of leaves, mm
5 000	5.7[a]	0.84[a]	0.63[a]	6.7[a]	1.1[a]	0.6[a]
8 000	6.4[ab]	1.13[b]	0.68[a]	7.9[a]	3.3[b]	2.0[b]
11 000	6.6[b]	1.25[c]	0.71[a]	9.4[b]	4.4[b]	2.9[b]
CV, %	4.90	2.99	19.27	6.45	15.80	19.01

Means followed by the same letter in the same column are not significantly different based on the Tukey test, P = 0.05.

Table 2. 'Muhzoto' shoot growth performances under different concentrations of coconut water added in MS medium containing 1.5 vitamin strength

Coconut water concentration, ml l^{-1}	Height of shoots, cm	Stem diameter, mm	Internodus length, cm	Number of leaves per shoot	Length of leaves, mm	Width of leaves, mm
0	6.3[ab]	1.04[a]	0.82[a]	7.6[a]	3.8[a]	6.0[c]
25	6.1[ab]	1.03[a]	0.72[a]	7.0[ab]	3.3[a]	59.8[b]
50	6.6[a]	1.18[a]	0.77[a]	7.2[ab]	4.2[a]	76.8[a]
75	6.3[ab]	1.12[a]	0.68[a]	7.1[ab]	3.8[a]	82.9[a]
100	5.7[b]	1.05[a]	0.79[a]	6.8[b]	3.7[a]	89.6[a]
CV, %	5.54	9.17	12.42	4.60	11.15	7.57

Means followed by the same letter in the same column are not significantly different based on the Tukey test, P = 0.05

Table 3. Shoot growth performances derived from 'Muhzoto' explants under different culture media

Culture medium, CM	Height of shoots, cm	Stem diameter, mm	Internodus length, cm	Percentage of airy roots per shoot, %	Average number of roots per nodus	Root length, cm	Number of leaves per shoot	Length-width leaf ratio	Percentage of shoots branched, %
CM-1	5.4[a]	0.85[ab]	0.62[a]	74.3[a]	1.5[a]	1.4[a]	6.7[a]	1.40[a]	56.7[a]
CM-2	5.3[a]	1.13[ab]	0.67[a]	61.5[a]	1.6[a]	1.0[a]	7.5[a]	1.42[a]	50.6[a]
CM-3	3.9[a]	0.71[b]	0.53[a]	54.5[a]	1.3[a]	1.1[a]	6.6[a]	1.40[a]	17.9[b]
CM-4	5.3[a]	1.23[a]	0.71[a]	62.7[a]	1.6[a]	1.3[a]	7.3[a]	1.43[a]	45.2[a]
CV, %	15.25	15.56	17.67	12.21	19.94	17.82	11.19	8.95	18.24

Notes: Culture media (CM) of MS medium supplemented with 1.5 vitamin strength in the medium in combination with 100 mg l^{-1} myoinositol, 1 mg l^{-1} calcium pantothenate (CaP), and 0.1 mg l^{-1} gibberellic acid-3 (GA3) (CM-1); 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP dan 0.1 mg l^{-1} GA3 (CM-2); 1 mg l^{-1} CaP and 100 ml l^{-1} CW (CM-3); and MS medium supplemented with 1.5 total vitamin strength in the medium (CM-4 as control). Means followed by the same letter in the same column are not significantly different based on the Tukey test, P = 0.05.

Shoot growth performances of 'Muhzoto' explants under different concentration of coconut water (CW)

Different concentrations of CW added in MS medium containing 1.5 total vitamin strength in the medium could not give a positive effect on shoot growth performances derived from 'Muhzoto' explants. Adding of different CW concentrations was due to causing high explant contamination. Higher concentration of CW added in the medium, higher explant contamination recorded (Table 2). Shoots derived from the CW treatments were generally having greener and strong stems, darker green leaves and easily grown white friable callus in the bottom position of nodes. Though all CW treatments still successfully induced shoots, all regenerated shoots were generally not suitable to be subcultured in producing qualified planting materials. Optimal shoot growth performances kept noted in 'Muhzoto' explants cultured on MS medium containing 1.5 vitamin strength (Fig. 1B). The treatment stimulated healthy shoots with 6.3 cm in shoot height; 1.04 mm in stem diameter; 0.82 cm in internodus length; 7.6 leaves per shoot; 3.8 mm in leaf length and 6% of contamination.

Shoot growth performances of 'Muhzoto' explants under different culture media

Different culture medium supplemented with adding myo-inositol, CaP, GA3 with 3 in subscript position, and CW in MS medium gave different results. Culture medium stimulating better shoot growth performances was recorded on CM-2 (MS medium containing 200 mg l^{-1} total myoinositol in the medium, 1 mg l^{-1} CaP and 0.1 mg l^{-1} GA_3). The medium induced greener shoots with 1.13 mm stem diameter and the high number of leaves per shoot up to 7.5 leaves (Fig. 1C). Similar results with better stem diameter up to 1.23 mm, longer internodus and a lower percentage of shoots branched of 45.2% were noted on 'Muhzoto' explants cultured on MS medium containing 1.5 vitamin strength as control (CM-4). The lowest results were determined on explants cultured on CM-3 (MS medium added by 100 mg l^{-1} myoinositol, 1 mg l^{-1} CaP, 100 ml l^{-1} CW and 0.5 vitamin strength. Though the treatment had the lowest percentage of shoots branched down to 17.9%, shoots regenerated from the medium generally had shorter and tinner shoots compared to others. Shoots derived from the treatment were also not suitable for producing good planting materials.

Shoot growth response study of 'Muhzoto' explants

Interesting phenomenons were successfully revealed in the fourth experiments when we tried to cluster different explants derived from regenerated 'Muhzoto' shoots and cultured them on a selected medium *i.e.* MS medium supplemented with 1.5 vitamin strength. Shoot tip explants produced shoots with high shoots, 0.82 cm internodus length and percentage of shoots branched up to 24.3% (Table 4; Fig 1D). First nodus explants

stimulated shorter shoots with the highest shoots branched as high as 95.3% (Fig. 1E), followed by the 2nd nodus explant with 71.1% branched shoots (Fig. 1F). Percentage of shoots branched declined on 3rd nodus explant (Fig. 1G) with the lowest percentage of shoots branched down to 6.2% and good quality of shoot performances, homogeneity, vigour and healthy noted on shoots derived from 4th nodes (Fig. 1H). While 5th nodus explants had different responses compared to others. The explants generally induced shoots with varied performances (Fig. 1I). From the experiments, it was revealed that there were different explant responses regenerated from different 'Muhzoto' explants and two different clusters of explants were categorized. The shoot tips, 1st and 2nd nodes generally induced higher results on length of internodus with a high percentage of branched shoots, while 3rd, 4th and 5th stimulated higher stem diameter and the number of leaves per shoot with a low percentage of shoot branched.

Table 4. Shoot growth behaviour of 'Muhzoto' explants cultured on MS medium supplemented with 1.5 vitamin strength

Explant type	Shoot height, cm	Diameter of stems, mm	Length of internodus, cm	Number of leaves per shoot	Percentage of shoots branched, %
Shoot tips	4.8[a]	0.81[c]	0.82[a]	5.1[b]	24.3[c]
1st nodes	3.4[c]	0.82[c]	0.61[a]b	5.0[b]	95.3[a]
2nd nodes	3.6[c]	1.04[b]	0.64[a]b	5.3[b]	71.1[b]
3rd nodes	3.7[c]	1.19[b]	0.46[b]	6.7[a]	9.2[d]
4th nodes	4.1[b]	1.49[a]	0.68[a]b	6.5[a]	6.2[d]
5th nodes	3.7[c]	1.05[b]	0.55[b]	6.6[a]	10.2[d]
CV, %	3.08	5.35	1329	4.25	4.23

Means followed by the same letter in the same column are not significantly different based on the Tukey test, P = 0.05

In a further study, subculturing 3rd and 4th nodes generally induced healthy and vigorous shoots with high homogeneity. Though the 5th nodes had lower results compared to the 3rd and 4th nodes, the nodes were still able to regenerate better shoot growth performances than the shoot tips, 1st, and 2nd nodes. While subculturing the branched shoots derived from the shoot tips, 1st and 2nd nodes continuously till two to four times generally resulted in hairy shoots with thin stems, high airy roots, small and undeveloped leaves (Fig. 1K). The low quality of regenerants usually occurred when the branched shoots did not handle properly during subculture, however, optimal handling of the shoots followed by selecting and applying the right time of shoot subculturing could still maintain the quality of regenerated shoots up to next twice to fourth subcultures (Fig. 1L). From the experiment, it was revealed that the branched shoots were important and significant problems in *in vitro* shoot proliferation of 'Muhzoto' explants vegetatively (Fig. 1J). Therefore, for the 'Muhzoto' variety, producing high quality *in vitro* planting materials could be carried out primarily by subculturing 3rd, 4th and 5th nodes or subculturing the shoot tips, 1st and 2nd nodes with optimal handling. The optimal handling carried out by choosing, selecting and applying the right time of subculture of the 3rd, 4th and 5th nodes were resulted in high quality in vitro planting materials and maintained maximal growth of branched shoots derived from shoot tips, 1st and 2nd nodes till next two to four subcultures.

Different shoot growth performances due to different treatments on 'Muhzoto' explants were successfully revealed from the study. In the experiment, higher light intensity, well and healthy shoots derived from 'Muhzoto' explants were established. High light intensity up to 11 000 lx applied continuously at 24 ± 2 °C stimulated greener and healthy shoots up to 6.6 cm in height, 1.3 mm in stem diameter, 0.71 cm in internodus length, 9.4 number of leaves per shoot, 4.4 mm in leaf length and 2.9 mm in leaf width. In other studies, maintaining culture in culture room at 25 ± 2 °C on 16 h illumination of daylight fluorescent tube lamps with 2 000 lx light intensity induced shoot formation up to 51.2% for 'Daraga' variety using medium protocol C (Al-Sulaiman, 2011). Incubating cultures in a growth chamber at 25 ± 2 °C under a photoperiod of 16/8 h with 3 335 lx using Philips white fluorescent lamps resulted in shoots with 9 cm in height and 9 leaves per shoot (Ebad *et al.*, 2015). Incubating cultures at 25 ± 2 °C under a photoperiod of 16/8 h with 2299 lx using Philips white fluorescent tubes stimulated shoot regeneration up to 96% on 'Diamant' variety (Kaur, 2015). Growth chamber at 25 °C providing 16 h photoperiod of 200 lx light intensity was significantly for producing shoots with 10.3 cm shoot height and 7.4 number of nodes per plant for 'Desiree' variety (Mehmood *et al.*, 2016). Growth chamber with 22-watt LED rod light (± 8 000 lx) in red successfully stimulated the growth of 'Cardinal' shoots with 7.5 cm shoot height, 8.6 number of leaves per shoot, 6.8 number of nodes per shoot and 3.8 roots per shoot (Karmakar *et al.*, 2018).

The utilization of CW to improve the growth of explants was successfully applied in *in vitro* culture of several plants published previously. Adding 10% of CW in half-strength MS medium gave a positive effect in improving the growth of *Dendrobium* plantlets compared to VW, KC and NP medium (Aktar *et al.*, 2008). The treatment induced the height of plantlets up to 1.04 cm with 2.12 leaves per plantlet and 0.31 cm length of leaves.

Maximum shoot length (7.2 ± 0.16), number of shoots (11.5 ± 1.5) and number of nodes (4.6 ± 0.22) were achieved on the MS medium containing 20% (v/v) coconut water with 2.0 mg l^{-1} of BAP for Kiwifruit (*Actinidia deliciosa*) (Nasib *et al.*, 2008). Modified Hyponex media (Hyponex 20N:20P: 20K and 6.5N: 4.5P:19K, 1 g l^{-1}) containing 30 ml l^{-1} CW successfully

induced better fresh dan dry weight of *Calanthe* hybrids 'Bukduseong' × 'Hyesung' plantlets with 9.1 cm shoot height, 1.2 cm leaf width, 7.2 cm number of roots per plantlet and 1.8 cm^2 leaf area; while the similar medium supplemented with 50 ml l^{-1} CW stimulated better growth of *Calanthe* hybrids 'Chunkwang' × 'Hyesung' plantlets (Baque *et al.*, 2011). The best medium for growing and developing seedlings to become fully expanded plantlets was determined on half strength of Murashige and Skoog medium supplemented with 40% (v/v) CW (Hasan *et al.*, 2011). Medium CM5 (one-fourth MS medium containing 0.10 mg l^{-1} NAA, 0.70 mg l^{-1} KIN and 20 % coconut water) was the best in the propagation of *C. rubens* with 70% viability; 2.33 shoots; 47.33 mm shoot length; 36.33 mm root length and 65% callus formation (Gbadamosi, Sulaiman, 2012). Those studies revealed that adding CW on medium had a positive effect on the development and growth of shoots, while in the study, application of different CW concentration caused reducing quality of shoot growth with high contaminations noted. All shoots derived from the treatments were not suitable used for subculturing materials in conjunction to produce qualified potato planting materials for *in vivo* activities.

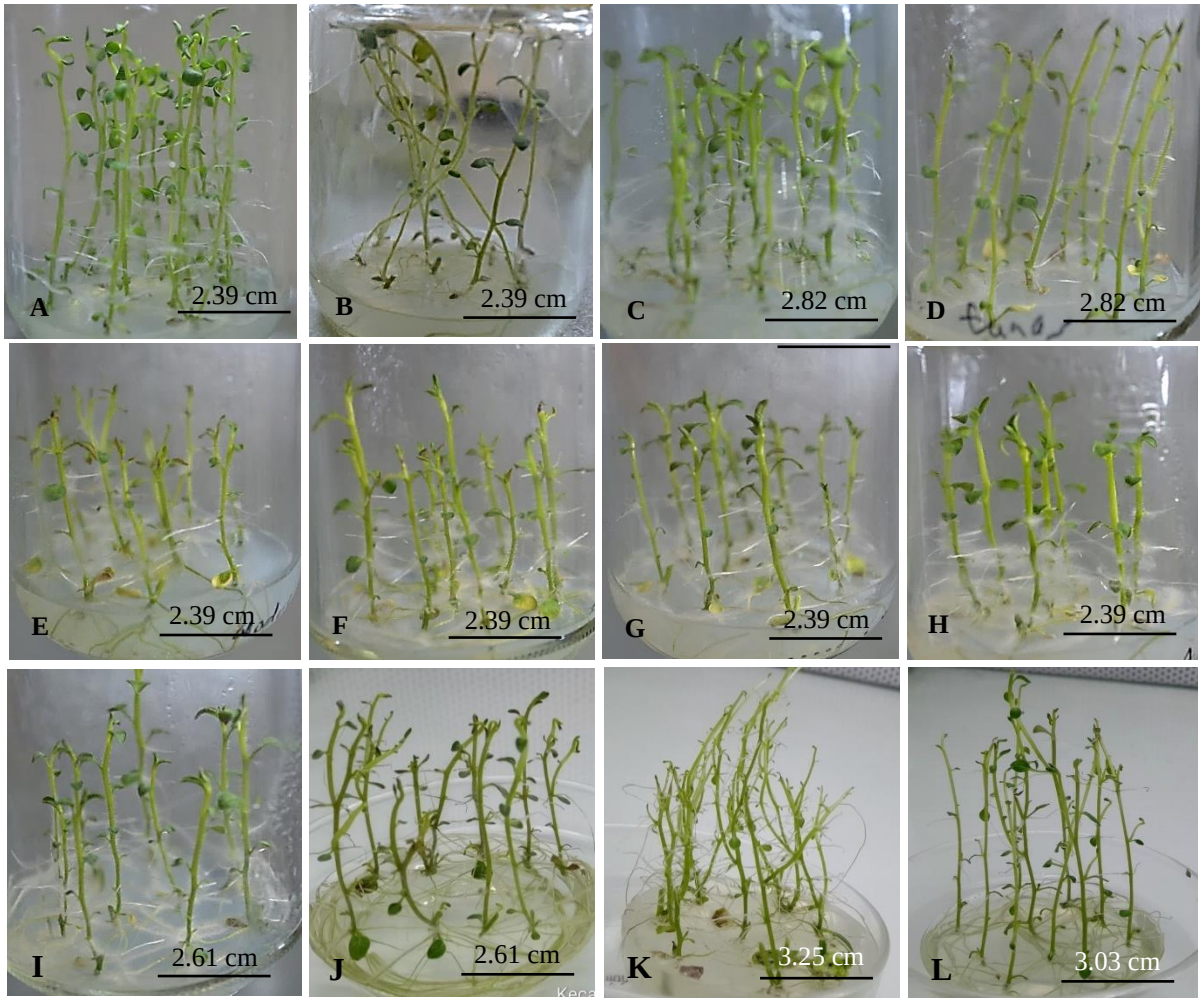

Figure 1. Shoot growth performances derived from 'Muhzoto' explants under different treatments. A. Morphological growth of shoots under 11 000 lx light intensity. B. Shoot growth performances under 0 ml l^{-1} CW in MS medium with 1.5 vitamin strength. C. Shoot performances of 'Muhzoto' explants cultured on MS medium containing 200 mg l^{-1} total myoinositol in the medium, 1 mg l^{-1} CaP dan 0,1 mg l^{-1} GA_3 (CM-2), D. Shoot growth behaviour derived from shoot tip explants, E. Shoot growth behaviour derived from 1st nodus explants, F. Shoot growth behaviour derived from 2nd nodus explants, G. Shoot growth behaviour derived from 3rd nodus explants, H. Shoot growth behaviour derived from 4th nodus explants, and I. Shoot growth behaviour derived from 5th nodus explants. J. The branched shoots derived from 1st nodes in the first culture 40 days after culture. K. The hairy shoots with thin stems, high airy roots, small and undeveloped leaves derived from the branched shoots of 1st nodes after the third subculture. L. Shoot growth performances derived from the branched shoots of 1st node explants after the 3rd subculture under optimal handling dealing with selection and application of the right time of subculture.

In *in vitro* culture, each step needs a suitable medium to obtain the optimal response of explant in different ways of regeneration methods. In the study, optimal shoot growth performances of 'Muhzoto' variety were established using MS medium enriched by 1.5 vitamin strength, 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP and 0.1 mg l^{-1} GA_3, though there was no significant difference compared to MS medium supplemented with 1.5 vitamin strength. The treatment produced shoots with 5.3 cm in height, 1.13 mm stem diameter, 0.7 cm

internodus length, 7.5 leaves per shoot, 1.42 length-width ratio and 50.6% shoots branched. From other studies it was reported that MS medium containing 0.5 mg l^{-1} BA and IBA was important to produce 2.5 shoots per explant with 5.43 cm shoot length, 100% shoots rooted and 15.8 roots per shoot (Nagib *et al.*, 2003). Ebad *et al.* (2015) successfully induced shoots with 8.3 cm in height and 3 leaves per shoot using MS-0 medium free plant growth regulator with 30 g l^{-1} sucrose and 8 g l^{-1} agar. Shoots with 7.1 cm in height, 7 leaves per shoot, 109.3 mg shoot fresh weight, 13.3 mg shoot dry weight and 12.2 dray matter were significantly regenerated using MS-0 medium hormone-free after 4 weeks of culture under mixo-trophic condition (Khalil *et al.*, 2016). Height of shoots up to 10.3 cm with 7.4 nodes per plant for 'Desiree' variety was strongly induced on MS medium supple-mented with 0.25 mg l^{-1} GA3; while for 'Cardinal' variety, shoots with 10 cm in height and 7.4 nodes per plant were established on MS medium containing 0.12 mg l^{-1} GA3 (Mehmood *et al.*, 2016)

Culture responses expressed on different typical shoot growths were noted in the study. Utilization of shoot tips, 1st and 2nd nodes as explant source resulted in an almost high percentage of shoots branched leading to produce low-quality planting materials, while 3rd, 4th and 5th nodes were suitable explant source utilized to regenerate high-quality planting materials for *in vivo* propagation vegetatively. The branched shoots were an important and serious problem in *in vitro* 'Muhzoto' variety propagation, especially when the shoots were not handling properly under continuous subculture processes. The hairy shoots with thin stems, high airy roots, small and undeveloped leaves were real evidence obtained after 2–4 periodical subcultures. Other studies explored shoot growth performances by culturing node explant on different treatments and determined that good quality of shoots was regenerated by application of sucrose than commercial sugar; ultrapure water than tap water, bacteriological and gelrite than agar (Venkatasalam *et al.*, 2013). Different responses of explant growth also revealed by culturing explants in two different incubation condition and different places of explant on culture media either horizontally, upright or inverted on culture media (Mng'omba *et al.*, 2017). Under different combination treatments, they found different growth responses of explants during *in vitro* culture. Placing explants in inverted position incubated in the dark condition resulted in high shoot length, shoot number, root number and leaf number. The second-best combination treatments were established on explants cultured horizontally and incubated in the dark then transferred to light incubation, while upright position explants cultured in all incubation condition gave low results in all variable observed.

Conclusion

From all treatments applied in the study, it can be concluded that the four different treatments explored gave different and interesting results. Higher light intensity up to 11 000 lx was the optimal treatment in resulting in better shoot growth performances of 'Muhzoto' explants. Adding different concentrations of CW could not improve the quality of shoots, but the treatment was due to leading on reducing shoot growth with high contamination occurred. Varied culture media that were enriched by adding myoinositol, CaP, GA3 and CW, did not increase shoot growth performances significantly, except CM-2 (MS medium supplemented with 1.5 vitamin strength in the medium in combination with 200 mg l^{-1} myoinositol, 1 mg l^{-1} CaP and 0.1 mg l^{-1} GA_3. Though the culture medium slightly improved shoot performances, however, there was no significant difference compared to the MS medium containing 1.5 vitamin strength. Furthermore, exploring shoot growth responses derived from different types of 'Muhzoto' explants successfully revealed that shoot tips, 1st and 2nd nodes generally regenerated high branched shoots with the higher length of internodus; while 3rd, 4th and 5th nodes successfully stimulated higher stem diameter and the number of leaves per shoot with a low percentage of shoot branched. The branched shoots were the serious problem in preparing high-quality regenerants derived from the 'Muhzoto' explants.

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Conflict of interest

We declare that there is no conflict of interest dealing with IAARD that funding this research activity, Kalimandi Main Institute for Horticulture Seeds (KMIHS), Banjarnegara District, Central Java province, Indonesia which supplied the research materials, authors, and Central Java Assessment Institute for Agriculture Technology that facilitated the research activities.

Author contributions

BW – contributed to research planning, executing till finishing, preparing and writing the manuscript until revisions of it.

IGC, RKJ, SCB and BH – were involved in preparing research materials, carrying research and helping data analysis equally. All authors approved the manuscript for publication, take public responsibility for the content.

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