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In Vitro Shoot Generation from Cotyledon Explant of *Brassica Oleracea* using BAP and NAA

Nazirwan

Seed Technology Study Program, Department of Food Crop Cultivation Lampung State Polytechnic

Abstract: ***Broccoli (Brassica oleracea) is an important vegetable crop grown in the highlands of Indonesia. Cotyledon is used as a source of explants for in vitro shoot regeneration. The objective of this research was to examine the influence of the growth regulators 6-benzyl amino purine (BAP) and naphthalene acetic acid (NAA) on shoot formation in these cultivars. The shoot regeneration system of cotyledoned explants can be useful as a tool for synthetic seed formation. Cotyledon explants excised from 14-day-old in vitro germinated seedlings were placed on shoot induction medium containing basal salts of Murashige and Skoog (MS) and various concentrations of BAP and NAA. The highest percentage of cotyledon explant producing shoot (90%) and the highest mean number of shoots produced each bottle of culture (1.15) were obtained on 1 mg/l BAP. Therefore, 2 mg/l BAP with 0.5 mg/l NAA are the recommended combinations for shoot regeneration from cotyledonary explants.***

Keywords: Broccoli, 6-benzyl amino purine, naphthalene acetic acid, in vitro, shoot formation, cotyledon

1. Introduction

Broccoli includes the main vegetables of the *Brassicacea* family, the main part of this plant used is flower buds. Broccoli is highly susceptible to high temperatures and grows best in cold climates, most of which are grown in Indonesia in the highlands. High temperature pressures can affect the vegetative and reproductive phases. The most popular method for the formation of in vitro shoots in the major vegetable crops of the *Brassicacea* family has been widely reported through organogenesis. Other micropropagation techniques, such as somatic embryogenesis, is less applied in this genus. Various tissues have been used in organogenesis of *Brassica* crops like hypocotyls, cotyledons and leaves (Javed *et al*., 2012), shoot tip (Abbas *et al*., 2012; Asim, 2012) thin layers of epidermal and subepidermal cells, roots, and protoplasts. Direct organogenesis has been achieved in a variety of *Brassica* species such as from the stem sections *of Brassica juncea* petioles of *Brassica napus*, hypocotyls of *Brassica napus* and cotyledonary explants of *Brassica compestris ssp. pekinensis*. *In vitro* regeneration is influenced by many factors such as culture environment, culture medium composition, explant source and genotype (Bano *et al*., 2010). *In vitro* regeneration is influenced by many factors such as culture environment, culture medium composition, explant source and genotype (Bano *et al*., 2010). The system is often used as a model for various physiological, biochemical, genetic and structural investigations in plants. Cotyledon explant is commonly used in plant tissue culture technique to stimulate shoot initiation and the combination of BAP and NAA has been applied for shoot regeneration in many species (Garshasbi *et al*., 2012). However, reports of in vitro shoot regeneration such as adventitious shoots from *Brassica oleracea* cotyledon explants were few and faced with limited success (Ravanfar *et al*., 2011). Therefore, the main objective of this study was to determine the effect of BAP and the effect of BAP and NAA combination on shoot formation in vitro broccoli by using explants from cotyledons.

2. Materials and Methods

Hybrid seeds of broccoli F-1 Lucky (Primasid) were sterilized for 15 minute in 10-20% Clorox solution while shaken. Rinse seed with sterile water 3 times to remove clorox remnants. Seeds are grown on germination media consisting of non-treated MS salt (Murashige and Skoog, 1962). Well expanded broccoli cotyledons from 1 4 -day-old *in vitro* germinated seedlings were cut and inoculated horizontally on the surface of medium. pH of media was adjusted to 5.7 prior to autoclaving at 121ºC, 1.03 kPa for 30 minutes. Eksplan was cultured in a 100 ml jam bottle containing 40 ml medium and sealed with aluminum foil. Cultures were maintained at 24ºC and 16 hours fotoperiod using cold white fluorescent lamps that gave intensity of 60 µmol m^{-2} s^{-1}. The concentrations of BAP were 1, 2 and 3 mg/l combined with NAA at 0 and 0.5 mg/l. Data on percentage of cotyledon explant producing shoots and mean number of shoots produced each bottle of culture were recorded after eight weeks of culture. Shoots were sub-cultured to fresh medium every four weeks. One pair of cotyledon explants planted for each culture bottle required five culture bottles for each treatment prepared in a Completely Randomized Duplicate (CRD) with 2 replications. Data were analyzed statistically according to statistic program of spss ibm 24 and if there was a different treatment significantly followed by Duncan's double-distance test at 5% level.

3. Results and Discussion

Cotyledon segments of broccoli were placed on MS medium containing different concentrations and combinations of BAP and NAA. The explants began to expand after three weeks of culture. After 8 weeks, significant differences were observed between the treatments on percentage of explants forming shoot (Fig.1). The highest percentage of shoot formation (90%) was in treatment containing 2 mg/l BAP and 0.5 mg/l NAA. It showed significant difference to the rest of the treatments except with 1 mg/l BAP. Meanwhile no significant difference in percentage of explants with shoots was observed between 1 mg/l BAP + 0.5 mg/1 NAA and 3

mg/1 BAP + 0.5 mg/1 NAA with 2 mg/1 BAP and 3 mg/1 BAP. The highest mean number of shoots per cotyledon explant (1.15) of broccoli was obtained in treatment containing 1 mg/l BAP (Fig. 2) which no differed significantly from the other treatments.

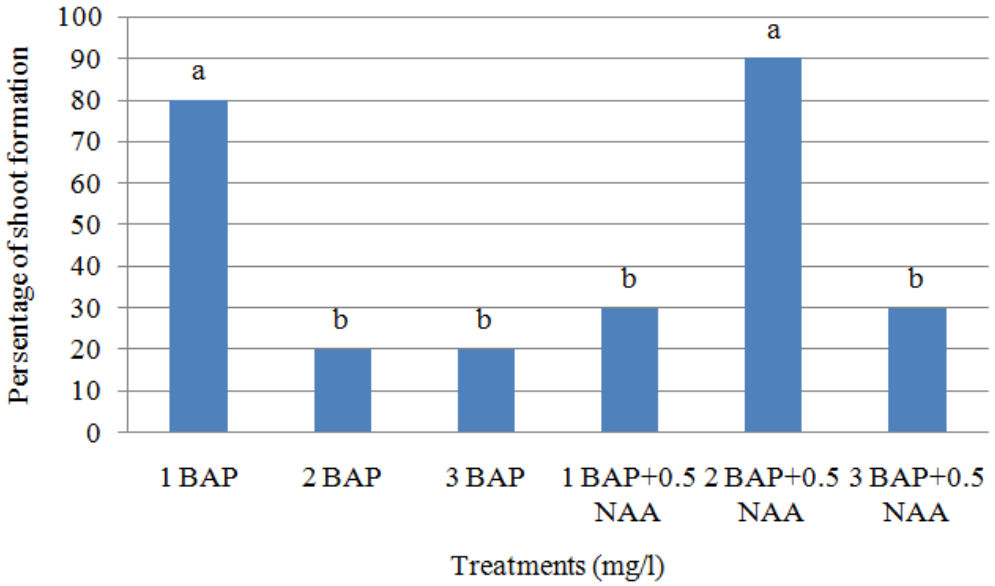

Figure 1: Effect of different concentrations of BAP in combination with NAA on percentage of cotyledon explants of broccoli shoots after eight weeks of culture. Means with the same letter were not significantly different at 0.05 probability level according to Duncan's double-distance test.

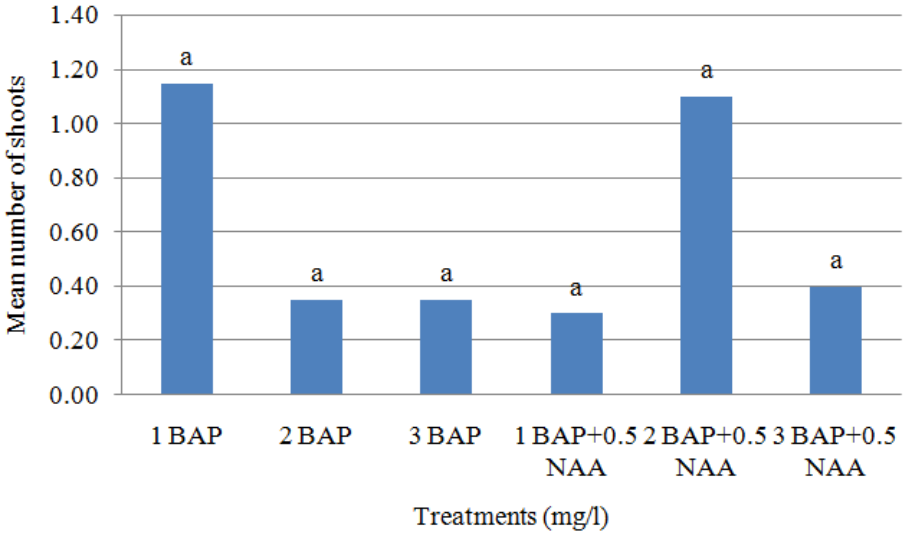

Figure 2: Effect of different concentrations of BAP in combination with NAA on mean number of shoots produced per cotyledon explant of broccoli after eight weeks of culture. Means with the same letter were not significantly different at 0.05 probability level according to Duncan's double-distance test

Fig. 3. Shows shoot formation from cotyledon explants of broccoli on MS medium containing different concentration of BAP with NAA. In this study, the wide range of BAP concentration used, with or without NAA, influenced shoot proliferation on cotyledon explants of broccoli. Increased concentrations of BAP alone from 1 mg/l to 2-3 mg/l BAP caused low proliferation of shoots in broccoli (Fig. 1). The best treatment on percentage of shoot formation from cotyledon explants of broccoli was 2 mg/l BAP with 0.5 mg/l NAA, producing 90 % shoot formation whereas the best treatment on mean number of shoot of broccoli was 1 mg/l BAP, producing 1.10 shoots per explant. BAP (1-3 mg/l) alone or in combination with NAA (0.5 mg/l) stimulated shoot formation of broccoli and also enhanced shoot elongation (Fig. 3, F). BAP at high concentration (2-3 mg/l) reduced shoot multiplication and BAP at 2 mg/l with 0.5 mg/l NAA, besides enhancing shoot multiplication, also trigged shoot elongation in broccoli. This study shows that the use of BAP in an appropriate ratio with NAA increased shoot formation from cotyledon explants of broccoli. Ravanfar *et al.*, (2011), BAP gave the highest number of shoots per hypocotyl and cotyledon segment with or without NAA on different cultivar of broccoli (*Brassica oleracea* subsp. *italica* cv. Green Marvel). Varied responses could be due to genotypic differences of the cultivar or plants reacting differently during micropropagation.

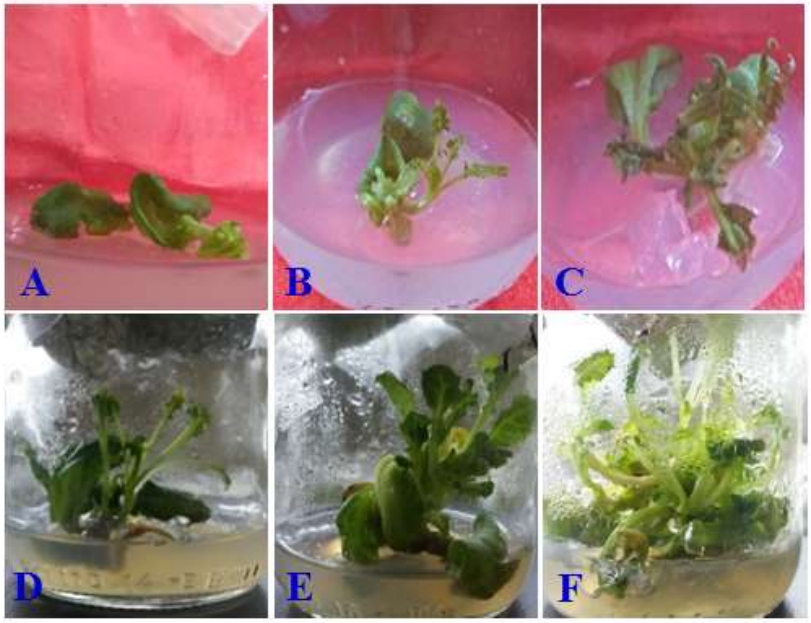

Figure 3: Multiple shoot formation from cotyledon explants of broccoli on different concentrations of BAP in combination with NAA. A (2 mg/l BAP), B (1 mg/l BAP) and C (3 mg/l BAP) after four weeks of culture with D (3 mg/l BAP + 0.5 mg/l NAA), E (2 mg/l BAP + 0.5 mg/l NAA) and F (1 mg/l BAP + 0.5 mg/l NAA) after eight weeks of culture

The few shoot formation on media containing 0.5 mg/l NAA in the experiments on broccoli could be due to the auxin having a more important role in cell division and callus formation. Nevertheless, the presence of a cytokinin, which in this study was BAP enhanced shoot formation as has been reported in often researches (Rafat *et al*., 2010).

4. Conclusion

The study showed that the cotyledon explants of broccoli cultivars were potential explants for *in vitro* shoot regeneration. The use of BAP as a cytokinin and NAA as an auxin in an appropriate ratio was most essential for shoot induction and multiplication from the cotyledon explants. The media containing only BAP caused explant swelling and produced fewer shoots. BAP at 2 mg/l with 0.5 mg/l NAA were the recommended combinations for shoot regeneration from cotyledon explant of broccoli.

5. Acknowledgements

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