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IN VITRO SHOOT GENERATION FROM COTYLEDON EXPLANT OF *BRASSICA OLERACEA* USING KIN AND NAA

Nazirwan

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

ABSTRACT

Cotyledon were used as explants in *in vitro* plant regeneration of broccoli (*Brassica oleracea*). The objective of this research was to examine the influence of the growth regulators 6- *Furfuryl amino purine* (KIN) 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 KIN and NAA. The highest percentage of cotyledon explant producing shoot (70%) and the highest mean number of shoots produced per cotyledon explant (1.80) were obtained on 2 mg/l KIN with 0.5 mg/l NAA. Therefore, 2 mg/l KIN with 0.5 mg/l NAA are the recommended combinations for shoot regeneration from cotyledonary explants.

Key word: *Broccoli, 6-Furfuryl Amino Purine, naphthalene acetic acid, in vitro, shoot formation, cotyledon.*

INTRODUCTION

In vitro regeneration offers a great opportunity for a rapid production of desirable and essentially genetically identical plants. An efficient *in vitro* regeneration system is also a crucial tool in genetic engineering of the crop for improved characteristics. Broccoli is one of the many valuable *Brassica* species, which is still less cultured under *in vitro* condition. 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. This paper reports on the influence of KIN either singly or KIN combination with NAA, on shoot formation from cotyledons explant of broccoli.

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. Well expanded broccoli cotyledons from 14-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 24 ± 2ºC and 16 hours fotoperiod using cold white fluorescent lamps that gave intensity of 60 µmol m^{-2} s^{-1}. The concentrations of KIN 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.

RESULTS AND DISCUSSION

Cotyledon segments of broccoli were placed on MS medium containing different concentrations and

combinations of KIN 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 (70%) was in treatment containing 2 mg/l KIN + 0,5 mg/l NAA. It showed significant difference to the rest of the treatments except with 2-3 mg/l KIN and 2 mg/l KIN + 0,5 mg/l NAA. Meanwhile no significant difference in percentage of explants with shoots was observed between 1 mg/l KIN + 0.5 mg/1 NAA and 3 mg/1 KIN + 0.5 mg/1 NAA with 2 mg/1 KIN and 3 mg/1 KIN. The highest mean number of shoots per cotyledon explant (1.80) of broccoli was obtained in treatment containing 2 mg/l KIN + 0,5 mg/l NAA (Fig. 2) which no differed significantly from the other treatments. Fig. 3. Shows shoot formation from cotyledon explants of broccoli on MS medium containing different concentration of KIN with NAA. In this study, the wide range of KIN concentration used, with or without NAA, influenced shoot proliferation on cotyledon explants of broccoli. Increased concentrations of KIN alone from 2 mg/l to 3 mg/l KIN and from 2 mg/l KIN + 0,5 mg/l NAA to 3 mg/l KIN + 0,5 mg/l NAA caused low proliferation of shoots in broccoli (Fig. 1).

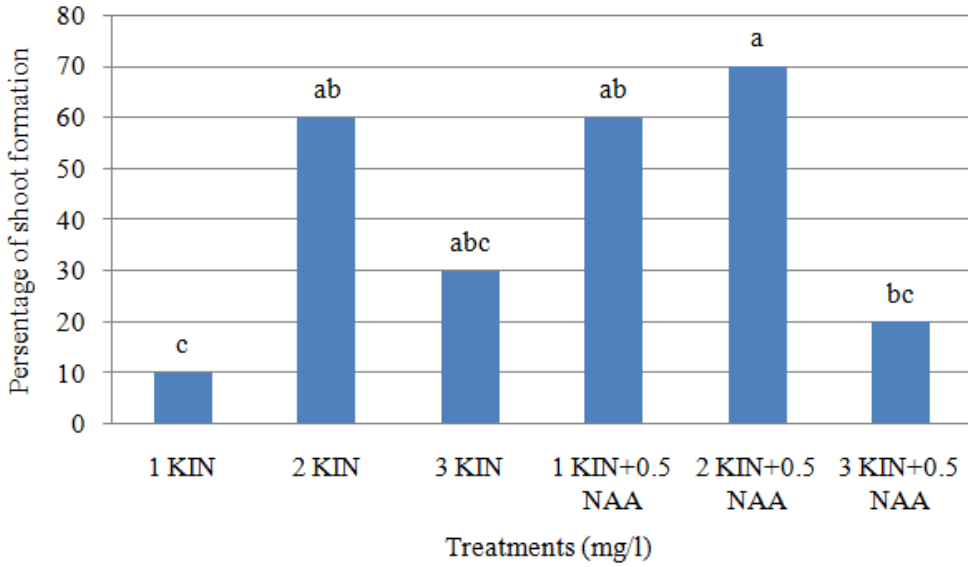

Fig. 1. Effect of different concentrations of KIN in combination with NAA on percentage of cotyledon explants of broccoli shoots after eight weeks of culture. Means with the same letter were not significantly different at 0.05 probability level according to Duncan's double-distance test.

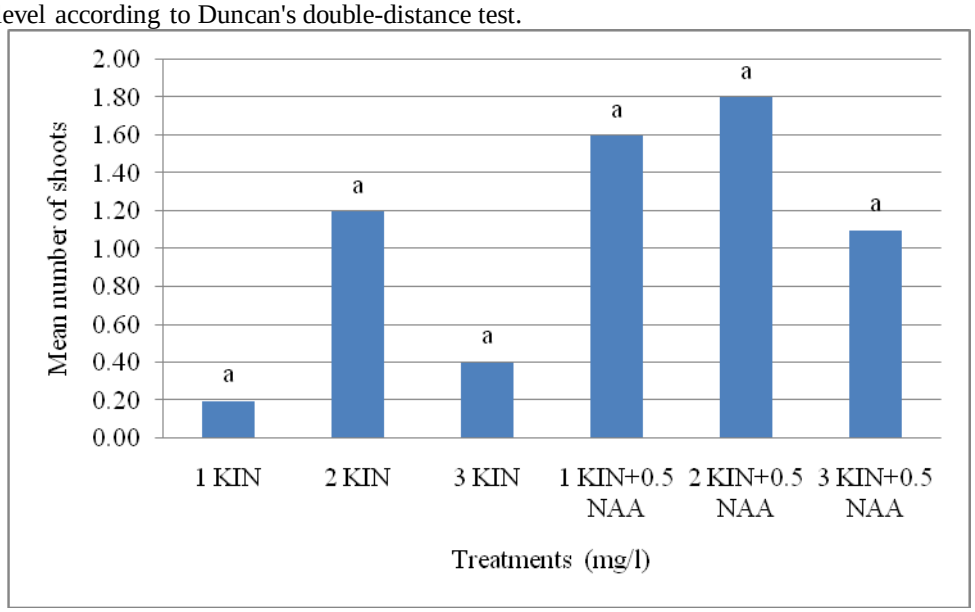

Fig. 2. Effect of different concentrations of KIN in combination with NAA on mean number of shoots produced per cotyledon explant of broccoli after eight weeks of culture. Means with the same letter were not significantly different at 0.05 probability level according to Duncan's double-distance test.

The best treatment on percentage of shoot formation from cotyledon explants of broccoli was 2 mg/l KIN + 0,5 mg/l NAA, producing 70 % shoot formation whereas the best treatment on mean number of shoot of broccoli was 2 mg/l KIN + 0,5 mg/l NAA, producing 1.80 shoots per explant. KIN (1-2 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).

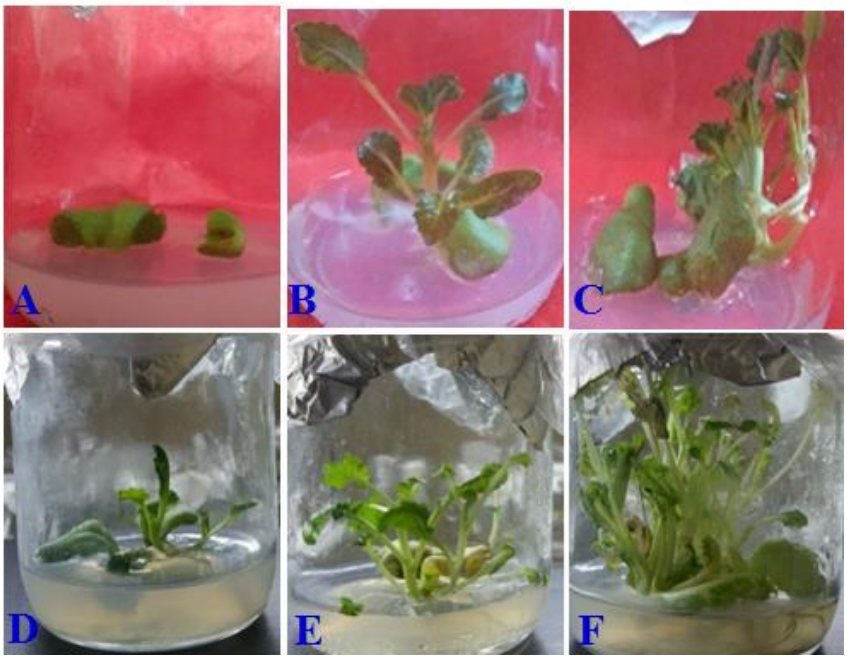

Fig. 3. Multiple shoot formation from cotyledon explants of broccoli on different concentrations of KIN in combination with NAA. A (2 mg/l KIN + 0.5 mg/l NAA), B (1 mg/l KIN + 0.5 mg/l NAA) and C (3 mg/l KIN + 0.5 mg/l NAA) after four weeks of culture with D (1 mg/l KIN), E (2 mg/l KIN) and F (2 mg/l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

KIN at high concentration (3 mg/l) reduced shoot multiplication and KIN 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 KIN in an appropriate ratio with NAA increased shoot formation from cotyledon explants of broccoli. Experimental result, the addition of cytokinins, such as kinetin would enhance shoot proliferation and root formation.

CONCLUSION

The study showed that the cotyledon explants of broccoli cultivars were potential explants for *in vitro* shoot regeneration. The use of KIN 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 KIN produced fewer shoots. KIN at 2 mg/l with 0.5 mg/l NAA were the recommended combinations for shoot regeneration from cotyledon explant of broccoli.

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