CALLUS INDUCTION IN *AMARANTHUS TRICOLOR* AND *AMARANTHUS SPINOSUS*

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ABSTRACT

Amaranths are an agriculturally valuable crop, but tissue culture techniques for these species remain limited. A study was conducted at the Plant Tissue Culture Laboratory, Institute of Crop Science, College of Agriculture and Food Science from October to December 2009. This study sought to identify a combination of plant growth regulators (PGRs) to induce callus formation on hypocotyl segments of *Amaranthus tricolor* and *Amaranthus spinosus*. *Amaranthus* spp. calli were yellow to deep yellow with a few cultures exhibiting red pigmentation depending on the PGRs applied in the induction medium. PGRs also influenced the number of days before callus outgrowths became visible. Callus formation in both species was faster with a combination of 6-benzylaminopurine (BAP) and 2,4-dichlorophenoxyacetic acid (2,4-D) than with α-naphthaleneacetic acid (NAA). *A. spinosus* calli were induced in 10 days with Murashige and Skoog medium (MS) + 0.5 mg L^{-1} BAP + 0.5-1 mg L^{-1} 2,4-D; callus induction took 12.5 days in MS + 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} 2,4-D in *A. tricolor*. In both species, BAP treatments caused profuse callus growth, but *A. tricolor* favored NAA, while *A. spinosus* was more responsive to 2,4-D. The *A. tricolor* calli scored with profuse growth also had the greatest mass. For *A. spinosus*, the calli with the greatest mass formed in MS + 0.5 mg L^{-1} BAP + 5.0 mg L^{-1} NAA, but the larger calli formed in BAP + 2,4-D-containing media.

Key words: callus culture, hypocotyl, 2,4-dichlorophenoxyacetic acid

INTRODUCTION

Amaranth (*Amaranthus* spp.), a.k.a "kulitis", Chinese tampala or pigweed, is a member of the Amaranthaceae family (Tisbe and Cadiz, 1967) and an herbaceous and agriculturally important annual plant in Mexico, Central and South America, India and Africa. The genus *Amaranthus* includes over 60 species found across many parts of the world (Willis, 1973). Amaranths are commonly consumed as vegetables or grain crops and have high nutritional value due to the presence of lysine and calcium (Coimbra and Salema, 1994; Pant, 1983) and high amounts of riboflavin, ascorbic acid and vitamin E. Amaranths also produce secondary metabolites, particularly compounds like betalain and anthocyanin (Wink, 2000). Betalain, a natural pigment derived from tyrosine (Leathers et al., 1992), and anthocyanin, a flavonoid (Mazza and Miniati, 1993), are natural food colorants that exhibit antiradical and antioxidant effects. Anthocyanin also possesses anti-inflammatory, antibacterial/antiviral,

anticarcinogenic, antitumor-promoting and antioxidant properties (Corke et al., 2003). These nutritional and therapeutic values of amaranth have increased its potential as a crop.

Plant tissue culture in amaranths remains limited. While there are early plant regeneration studies, the techniques require refinements. Callus formation was successfully induced in *A. paniculatus* (Bagga et al., 1987), *A. caudatus*, *A. hypochondriacus*, *A. cruentus* and *A. hybridus* (Bennici et al., 1992 and 1997). Shoot formation and plantlet regeneration were also achieved in *A. paniculatus* (Arya et al., 1993). In amaranth, plant tissue culture may have some practical applications, *i.e*., it can be used for micropropagation of its related genotypes, male-sterile plants, and stress-resistant genotypes and to rescue genetic variation or even induce new varieties and select regenerated plants for increased production of protein or specific amino acids (Bennici et al., 1992; Bennici and Schiff, 1997). Furthermore, this approach can be utilized for future research focusing on its therapeutic properties and application as natural colorants for food. This study aimed to explore the effects of 6-benzylaminopurine (BAP), a-naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) on callus induction in *A. tricolor* and *A. spinosus* to improve tissue culture of amaranths.

MATERIALS AND METHODS

In vitro-germinated seedlings

Seeds of two *Amaranthus* species, *A. tricolor* (Acc. # 210 - Western Samar) and *A. spinosus* (Acc. # 069 – Marinduque), were sourced from the National Plant Genetic Resources Laboratory (NPGRL), Institute of Plant Breeding, College of Agriculture and Food Science, UP Los Baños, College, Laguna, Philippines. The seeds were pre-treated with 0.36% fungicide (Benlate® or Benomyl®) for 15 minutes and rinsed with tap water. Surface sterilization of seeds for both *Amaranthus* spp. followed that of Flores et al. (1982), using a 10% (w/v) calcium hypochlorite solution following the double sterilization procedure in 10 min-10 min and 15 min-15 min sequences. The seeds were rinsed four times with autoclaved distilled water after the first and second sterilization steps. The sterilized seeds were then germinated in Murashige and Skoog basal medium (MS) (Murashige and Skoog, 1962) with 2% (w/v) sucrose and 0.5% (w/v) Pronadisa agar.

Plant material, culture media, and culture conditions

Hypocotyl segments (5 mm) obtained from ten-day old *in vitro* germinated seedlings of *A. spinosus* and *A. tricolor* (Fig. 1A and B) served as explants. MS medium was solidified with 0.5% (w/v) Pronadisa agar and the pH of the media was adjusted to 5.6 before autoclaving at 15 psi for 20 minutes. The hypocotyl segments were placed on solid MS (Murashige and Skoog, 1962) basal medium supplemented with BAP (0.5 and 1.0 mg L^{-1}), NAA (1.0, 5.0 and 10.0 mg L^{-1}), and 2,4-D (0.5, 1.0 and 2.0 mg L^{-1}), singly or in combination. BAP and 2,4-D were added as required. The culture vessels contained 10 mL of the media and cultures were kept in a growth room at 27°C $\pm$ 1°C under 20 W m^{-2} continuous light supplied by cool white fluorescent lamps.

Callus weight and degree of call us formation

Calli that formed from hypocotyls for each treatment were weighed after 4 weeks of incubation using a digital Mettler balance. The weighing procedure was carried out by placing the weighing balance inside the laminar flow hood wherein each callus was taken out of the culture bottle and placed on sterile paper on top of the balance. The weight (g) for each callus was recorded. The degree of callus formation was scored based on the extent of growth of the callus cells: light (1), moderate (2), moderately profuse (3) and profuse (4) growth as recorded after 4 weeks of callus induction.

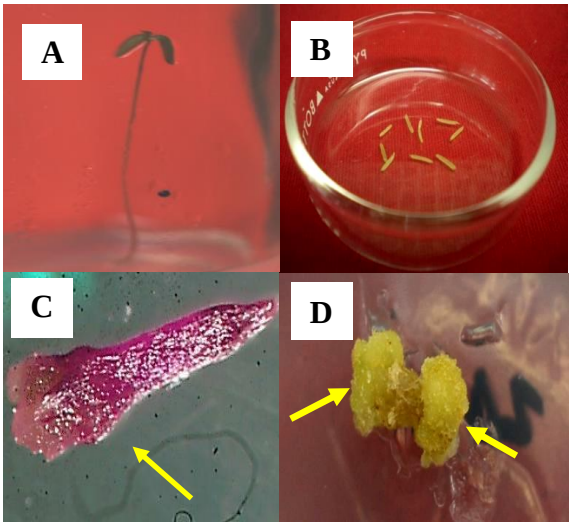


Fig. 1. **Culture initiation and callus induction. (A)** Representative ten-day old, amaranth seedling inoculated onto germination medium (MS basal salts). **(B)** Hypocotyl segments of 5 mm in length used as explants in subsequent experiments. **(C)** Swollen distal end of the hypocotyl (arrow) inoculated onto MS + 1.0 mg L^{-1}BAP. **(D)** Representative hypocotyl section of *Amaranthus* spp. inoculated onto MS + 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} 2,4-D. Calli formed at the proximal and distal ends (arrows).

Experimental design and data analysis

The experiment had a completely randomized design. Twenty samples were used for each treatment and the experiment was repeated twice. The data were analyzed using ANOVA through the Statistical Analysis Software (SAS) System (SAS Institute, Cary, NC, USA). Differences in treatment means were compared using the Duncan Multiple Range Test (DMRT) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Sterilization and germination of *Amaranthus* seed

Two amaranth accessions, *A. tricolor* (Acc. No. 210), a purple amaranth and *A. spinosus* (Acc. No. 069), a spiny amaranth, were used in this study. A total of 402 seeds of *A. tricolor*, and 166 seeds of *A. spinosus* were subjected to sterilization treatments (double sterilization for 20 or 30 minutes) and inoculated onto solid MS basal medium. Table 1 shows the percentage contamination, number of days to germination and percentage seed germination for both species per treatment. For *A. tricolor*, seeds sterilized for 10 min-10 min sequences gave 77.11% germination, while those subjected to a 15 min-15 min sterilization period gave 90.55%. The contamination rate was found to be 4.98% for the seeds treated to 10 min-10 min sterilization, and 3.98% for seeds treated to 15 min-15 min sterilization. Twenty-three percent (23.4%) of the total number of seeds inoculated did not germinate, which could be attributed to dormancy or loss of viability.

For *A. spinosus*, eighty-nine percent (89.16%) germination was attained from the seeds sterilized for 10 min-10 min, while 87.95% of the seeds germinated from the 15 min-15 min sterilization treatment. The remaining 11.44% of the total number of seeds could have been dormant or non-viable. The two accessions responded differently to the germination medium. For *A. tricolor*, the number of days to germination ranged from 6 to 25 for seeds sterilized for either 10 min-10 min or 15 min-15 min, whereas, for *A. spinosus*, the range was 3 - 8 days. Therefore, *A. spinosus* seeds were faster to germinate than *A. tricolor*.

Table 1. Response of two accessions of amaranth seeds to different durations of sterilization.

Treatments	Species	
	A. tricolor (n=402)	***A. spinosus*** (n=166)
10 min-10 min sequence		
% contamination	4.98	0
No. of days to germination	6 to 25	3 to 8
% seed germination	77.11	89.16
15 min-15 min sequence		
% contamination	3.98	0
No. of days to germination	6 to 25	3 to 8
% seed germination	90.55	87.95

Callus type and callus color

In this study, most calli in both *Amaranthus* species were compact and predominantly yellow (*A. tricolor;* Tables 2 and 4) or yellow-green with brown sectors (*A. spinosus*; Tables 3 and 4). However, there were regions in the calli that had either white, red, brown, light green, or yellow-green parts. Two of the four friable calli in *A. tricolor* were yellow, and the other two friable calli had yellow and brown sectors. Red and brown colored calli were observed in cultures with 10.0 mg L-1 NAA alone, or in cultures with equal doses of BAP and NAA or in BAP and 0.5 ppm 2,4-D. Friable *A. spinosus* calli were in varying degrees of yellow-green, white, or brown as seen for *A. tricolor*. Callus color may be an outcome of varying the PGRs in the callus induction medium (Yaacob et al., 2015). Yaacob et al. observed cream-colored calli produced from both leaf and stem explants of *A. cruentus* in 2,4-D and kinetin- or BAP-containing medium. A combination of GA_3 and zeatin also yielded cream-colored calli, while NAA and BAP produced cream and creamy-pink calli. However, after several weeks, only calli inoculated in BAP and 2,4-D changed to green—an indication of shoot initiation. A similar result was observed in the present study, particularly for *A. spinosus* calli, which turned green after four weeks. For *A. tricolor*, a change from yellow to green was only observed after four passages at 4-week intervals (16 weeks) in the same medium. However, shoot initiation was not observed throughout the experiment. Instead, somatic embryoids were noted (Fig. 4), which indicates that shoot formation could be achieved following an extended incubation period.

Table 2. Callus type and color of *A. tricolor* hypocotyl sections inoculated onto various media formulations consisting of MS basal medium added with BA, NAA and 2,4-D singly or in combination.

Type of Callus	Number of Callus Cultures	Number of Callus Culture (n=306)						
		Callus Color						
		Y[1]	**Y+W[2]**	**Y+R[3]**	**Y+B[4]**	**Y+R+B**	**Y+W+R**	**Y+W+R+LG[5]**
Compact	302	98	14	88	34	17	34	17
Friable	4	2	0	0	2	0	0	0

[1]Yellow, [2]White, [3]Red, [4]Brown, [5]Light green

Table 3. Callus type and color of *A. spinosus* hypocotyl sections inoculated onto various media formulations consisting of MS basal medium added with BA, NAA and 2,4-D singly or in combination.

Type of Callus	Number of Callus Cultures	Number of Callus Culture (n=219)				
		Callus Color				
		Y[1]	YG[2]	Y+B[3]	YG+B	YG+B+W[4]
Compact	214	2	17	29	125	41
Friable	5	0	0	0	2	3

[1]Yellow, [2]Yellow green, [3]Brown, [4]White

Callus induction and growth

Earlier reports have demonstrated that callus induction can occur from *Amaranthus* spp. hypocotyl segments and stem sections (Bagga et al., 1987; Bennici et al., 1992 and 1997). In a similar manner, *Amaranthus* spp. hypocotyl segments were used as explants in this study, but different species were tested. *A. tricolor* and *A. spinosus* hypocotyl segments were placed in MS-based media formulations with BAP (0.5, 1.0 mg L^{-1}), NAA (1.0, 5.0, 10.0 mg L^{-1}) and 2,4-D (0.5, 1.0, 2.0 mg L^{-1}) singly, or in combination (Fig. 1).

For both species, BAP in the media was not sufficient to promote callus formation (Figs. 2 and 3; Table 4). After four weeks, hypocotyl segments in MS medium devoid of PGRs turned reddish brown to dark brown, likely from phenolic oxidation (Figs. 2 and 3), and no callus formed. Several days after inoculation, explants in MS + 0.5 mg L^{-1} BA and 1.0 mg L^{-1} BA became swollen on either or both the proximal and distal ends of the hypocotyl sections, but also did not develop any callus. Meanwhile, media with a single synthetic auxin (2,4-D or NAA) was sufficient to promote callus growth in *A. tricolor*. This was not the case for *A. spinosus,* which required the presence of BAP in the medium along with 2,4-D or NAA to induce callus growth.

Callus outgrowths were first observed on either or both ends of the explant (Fig. 1C) after the ends became visibly swollen. Similarly, Singh et al. (2009) found that callus growth initiated on the surface or cut ends of explants during the *in vitro* propagation of sessile joyweed (*Alternanthera sessilis*), a member of the Amaranthaceae family. Likewise, in this study, extensive callus formation proceeded from the cut ends to the center of the hypocotyl segments until the entire explant became a mass of cells. Proliferative growth was sustained by regular transfer every four weeks to the same callus induction medium.

The synergistic effect of BAP and synthetic auxins on callus induction has been reported in *Amaranthus* spp., using either hypocotyl segments or stem sections with BAP and low doses of NAA or 2,4-D (Bennici et al., 1997; Guidea et al., 2012; Biswas et al., 2013). Bennici et al. (1992, 1997) reported the use of kinetin and 2,4-D with similar effectiveness in causing callus formation in hypocotyl segments. Further, the relative proportion of auxin and cytokinin in the media matters. In *A. gangeticus*, a higher BAP: NAA ratio was needed for optimal callus growth in stem explants (Amin et al., 2015), unlike in the present report where a lower cytokinin:auxin ratio was more suitable for *A. tricolor* and *A. spinosus*. The prior studies also reported differential morphogenic responses between *Amaranthus* species and breeding lines. This suggests that plant regeneration systems for *Amaranthus* may have to be developed independently, as genotype—in addition to the PGRs in the induction medium—has a pronounced influence on the *in vitro* response (Bennici et al., 1997).

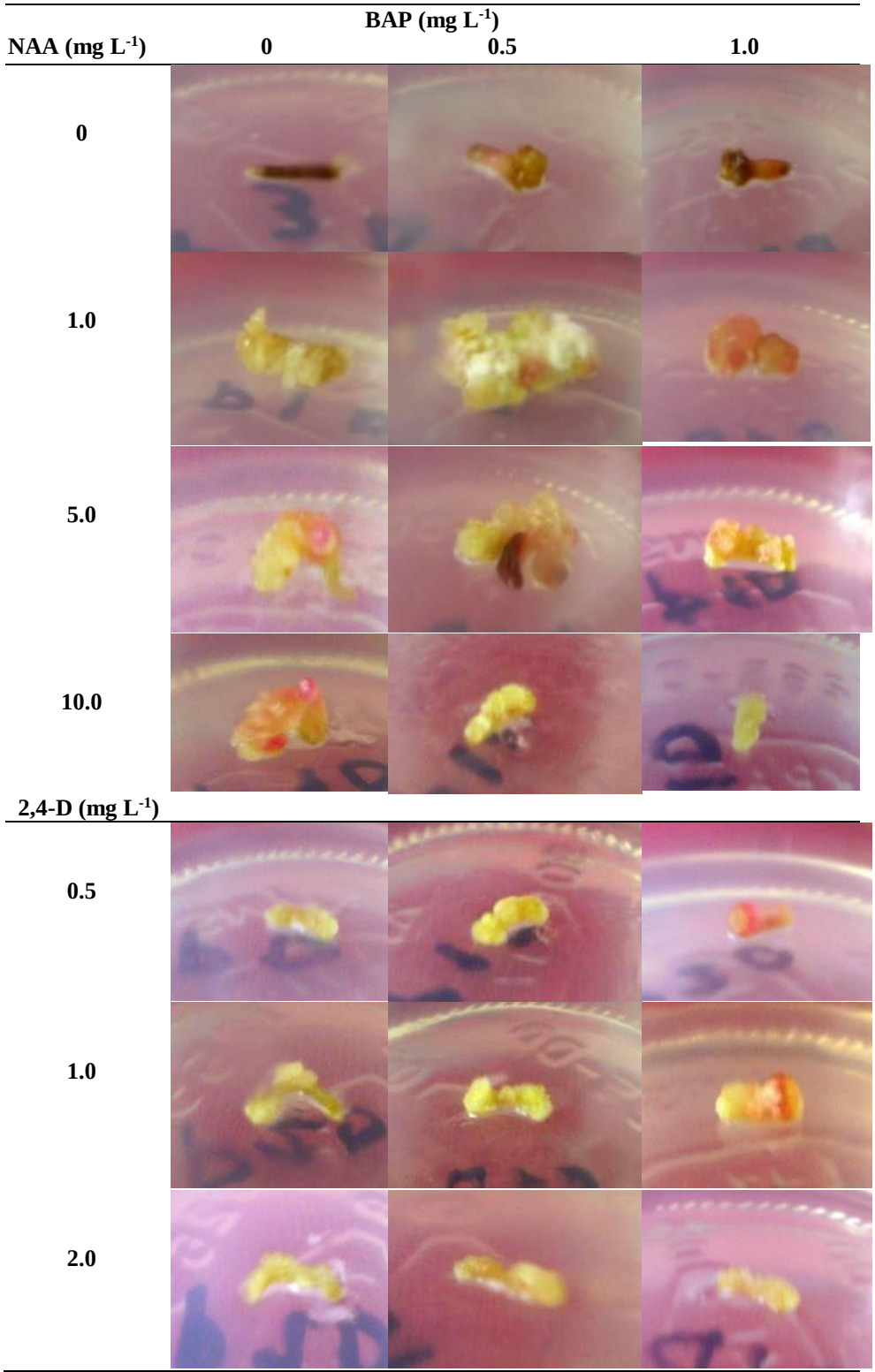


Fig. 2. Degree of callus formation and type of callus formed from *A. tricolor* hypocotyl sections inoculated in various media formulations four weeks after inoculation.

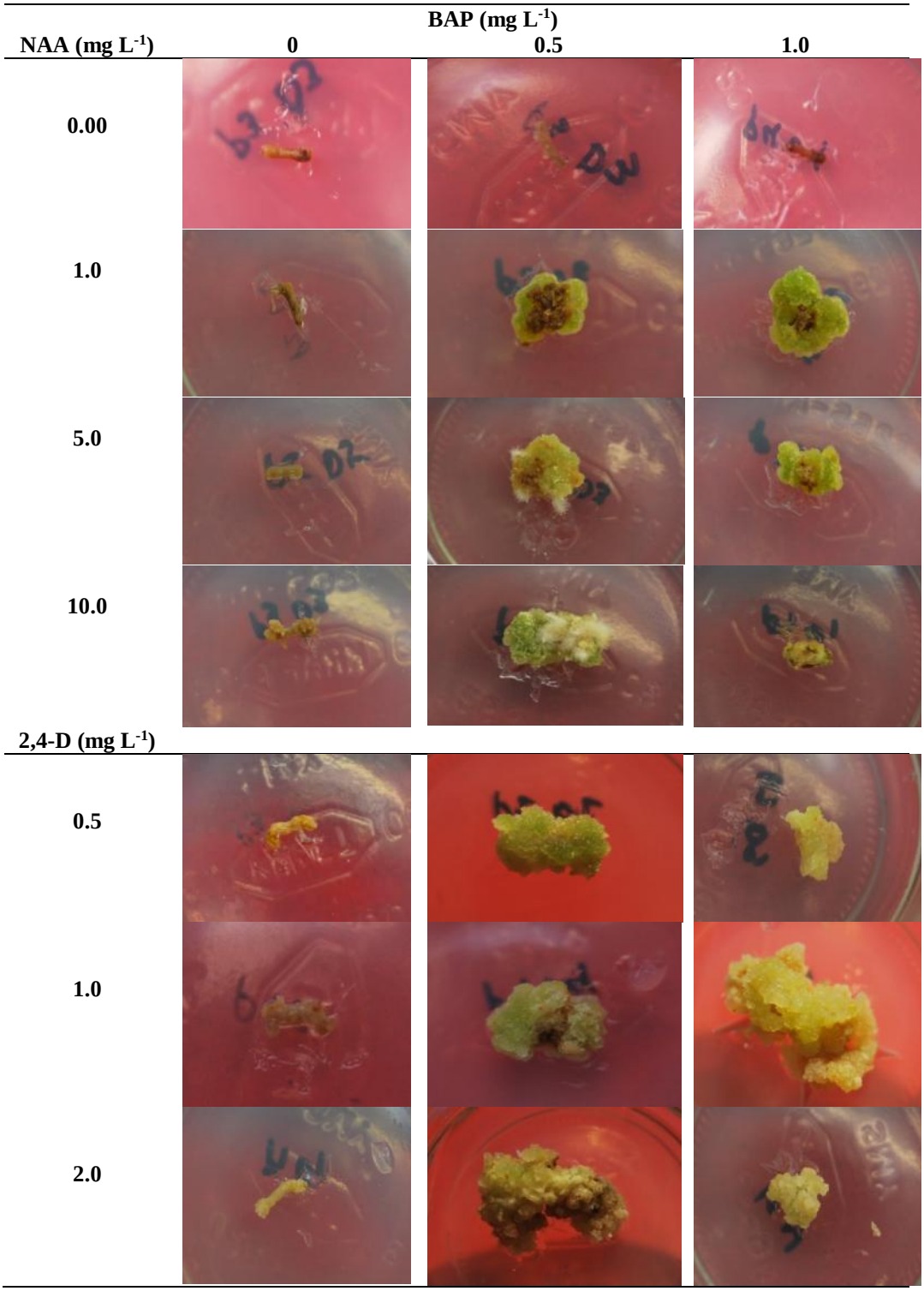


Fig. 3. Degree of callus formation and type of callus formed from *A. spinosus* hypocotyl sections inoculated in various media formulations four weeks after inoculation.

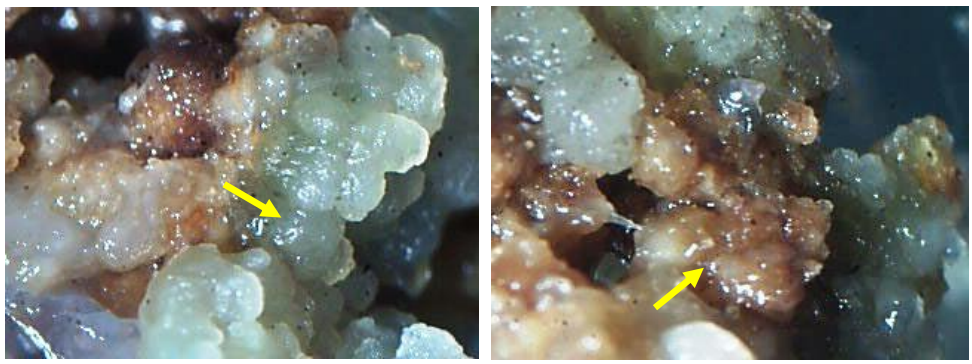


Fig. 4. Somatic embryo-like structures (left) that eventually turned brown (right).

Number of days to callus formation

The composition of the medium affected the time of callus initiation in addition to the color of the callus. Medium with 0.5 mg L^{-1} or 1.0 mg L^{-1} BAP in combination with any of the three levels of 2,4-D or NAA promoted earlier callus formation from hypocotyl segments of *A. tricolor* and *A. spinosus* than medium with 2,4-D or NAA alone (Fig. 5). If 2,4-D or NAA were added singly to the medium it took more than 30 days before callus formed in *A. tricolor*. Faster callus induction was possible in *A. tricolor* by combining 2,4-D or NAA and BAP, although the relative proportion of BAP and either auxin could vary. In general, 10.0 mg L^{-1} NAA or 0.5-1.0 mg L^{-1} 2,4-D and 0.5-1.0 mg L^{-1} BAP achieved the most rapid callus induction. However, while both 2,4-D and NAA were favored by *A. tricolor*, only 2,4-D with BAP yielded a shorter induction period for *A. spinosus*. In fact, auxins alone were unable to induce callus formation in *A. spinosus*.

Callus weight and degree of callus formation

For *A. spinosus*, the highest average callus weight (0.30 g) was obtained in cultures inoculated onto MS supplemented with 0.5 mg L^{-1} BAP + 5.0 mg L^{-1} NAA. More profuse growth, however, was recorded in 2,4-D containing media with BAP. In *A. tricolor*, the heavier calli grew in MS + 0.5 mg L^{-1} BAP + 10.0 mg L^{-1} NAA (2.54 g) and 1.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA (2.19 g). However, unlike in *A. spinosus*, the largest calli were also from the same treatment combination that gave the highest weight (Table 4).

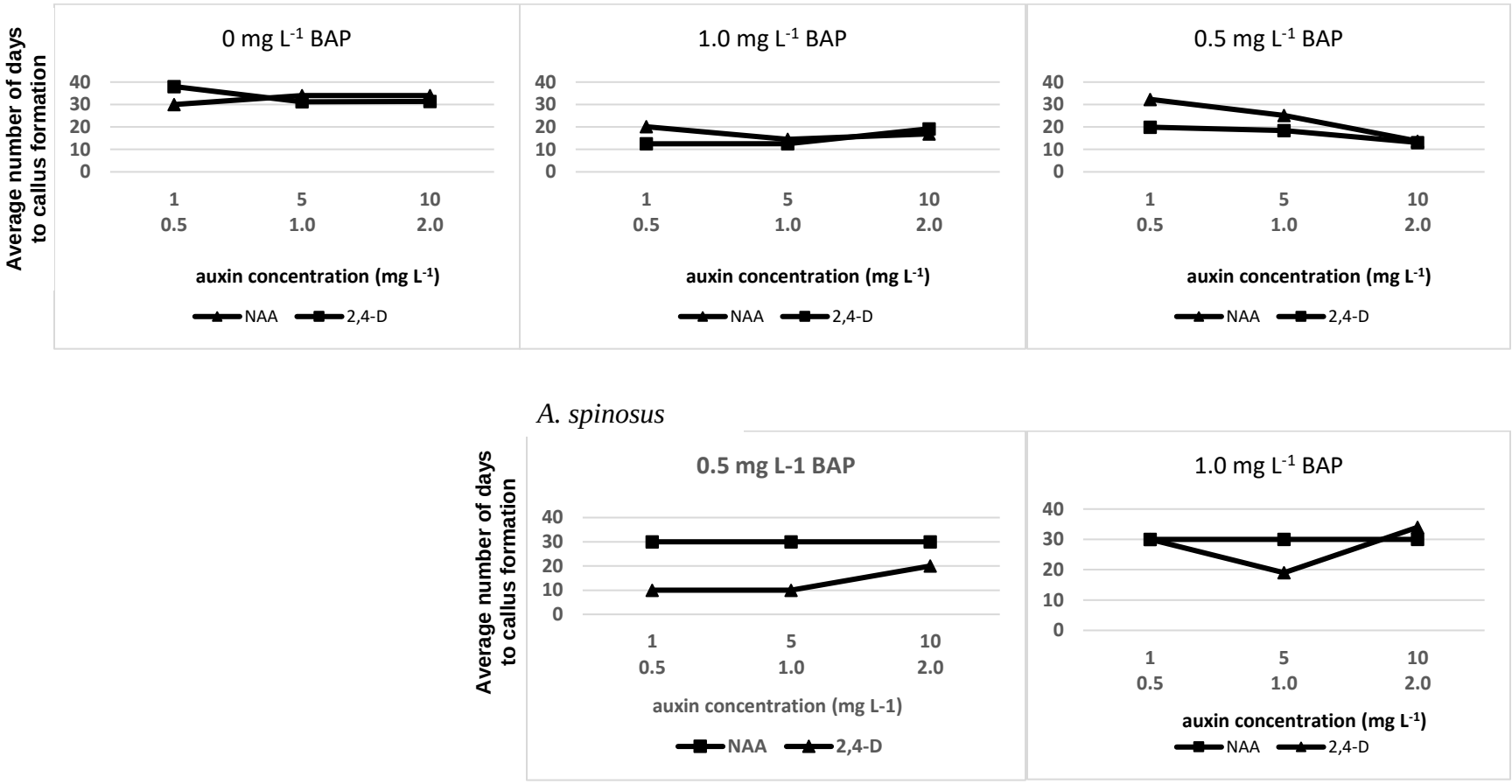
Root formation in hypocotyl explants

Root formation was observed in some hypocotyl explants of *A. tricolor* and *A. spinosus*, particularly near the distal end (Fig. 6). Root formation was observed in sections of *A. tricolor* inoculated onto MS alone or MS supplemented with 0.5 and 1.0 mg L^{-1} BAP alone or supplemented with 0.5 mg L^{-1} 2,4-D. Similarly, MS medium supplemented with proportional levels of BA and NAA (1.0 mg L^{-1}) induced root formation. Other media formulations did not induce root formation in the hypocotyl sections of *A. tricolor*. In contrast, all media formulations containing NAA singly or in combination with BAP at various concentrations induced root formation in hypocotyl sections of *A. spinosus*. Medium with the highest concentration of NAA (10.0 mg L^{-1}) combined with 1.0 mg L^{-1} BAP resulted in the highest number of *A. spinosus* root-forming hypocotyl cultures. These results indicate that NAA supplementation in the culture medium singly or in combination with BAP can induce root formation in *A. spinosus*, whereas MS basal medium alone induces root formation in *A. tricolor*. This result indicates that *A. tricolor* may have a sufficient level of endogenous auxin, and that exogenous application may have resulted in a supra-optimal concentration that led to the inhibition of root formation (Salisbury and Ross, 1992).

Table 4. Average callus weight and degree of callus formation in *Amaranthus* spp. in BA-containing media with varying levels of 2,4-D and NAA*.

TREATMENTS (mg/L)	A. Tricolor		A. spinosus	
	Average Callus weight (g) (n=40)	**Degree of Callus Formation** (n=40)	**Average Callus weight (g)** (n=40)	**Degree of Callus Formation** (n=40)
MS	-	-	-	-
MS + 0.5 mg/L BAP	-	-	-	-
MS + 1.0 mg/L BA	-	-	-	-
MS + 1.0 mg/L NAA	0.04 gh	1	-	-
MS + 5.0 mg/L NAA	0.15 efgh	1	-	-
MS + 10.0 mg/L NAA	0.31 defgh	2	-	-
MS + 0.5 mg/L 2,4-D	0.14 fgh	1	-	-
MS + 1.0 mg/L 2,4-D	0.46 defg	2	-	-
MS + 2.0 mg/L 2,4-D	0.02 h	1	-	-
MS + 0.5 mg/L BAP + 1.0 mg/L NAA	0.62 cd	2	0.15 c	2
MS + 0.5 mg/L BAP + 5.0 mg/L NAA	1.25 b	3	0.30 a	2
MS + 0.5 mg/L BAP + 10.0 mg/L NAA	2.54 a	4	0.12 cd	3
MS + 1.0 mg/L BAP + 1.0 mg/L NAA	0.36 defgh	2	0.24 b	2
MS + 1.0 mg/L BAP + 5.0 mg/L NAA	0.90 bc	3	0.08 def	2
MS + 1.0 mg/L BAP + 10.0 mg/L NAA	2.19 a	4	0.04 f	1
MS + 0.5 mg/L BAP + 0.5 mg/L 2,4-D	0.46 defg	2	0.08 def	3
MS + 0.5 mg/L BAP + 1.0 mg/L 2,4-D	0.35 defgh	2	0.07 ef	3
MS + 0.5 mg/L BAP + 2.0 mg/L 2,4-D	0.45 defg	2	0.23 b	4
MS + 1.0 mg/L BAP + 0.5 mg/L 2,4-D	0.58 cde	2	0.08 def	2
MS + 1.0 mg/L BAP + 1.0 mg/L 2,4-D	0.48 def	2	0.21 b	4
MS + 1.0 mg/L BAP + 2.0 mg/L 2,4-D	0.31 defgh	2	0.09 de	2

*Means followed with the same letters are not significantly different using DMRT at α=0.05.



Fig, 5. Average number of days to visible callus outgrowth as influenced by BAP, 2,4-D and NAA in *A. tricolor* (top panels) and *A. spinosus* (bottom panels). Callus did not form from *A. spinosus* hypocotyl explants without BAP. The values in the x-axis represent concentrations of NAA (upper) and 2,4-D (lower)

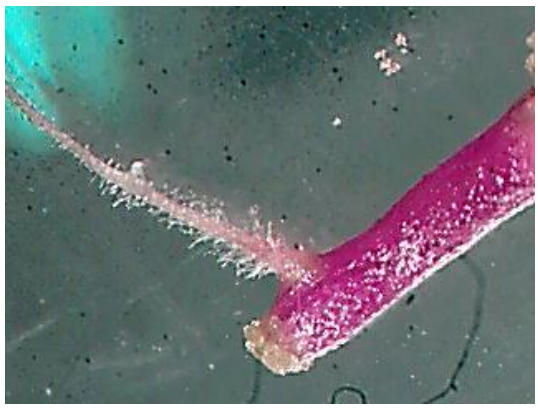


Fig. 6. Representative culture of hypocotyl section that formed callus at the distal end of the shoot inoculated onto MS + 1.0 mg L^{-1} NAA.

CONCLUSION

This study defined the combinations of plant growth regulators able to induce calli in *A. tricolor* and *A. spinosus*. Our results support earlier findings and add empirical evidence about the known effects of PGRs on callus induction in *Amaranthus* spp. The plant growth regulator combinations tested in this study did not induce shoots, and future research is necessary to explore the conditions needed to promote shoot formation and development. The differential varietal response observed in this study and in studies of other *Amaranthus* spp. indicates that genotype is a critical overriding factor influencing their *in vitro* morphogenic response. This suggests that independent plant regeneration systems are compulsory for *Amaranthus* spp.

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REFERENCES

Amin, M. A. M., N. A. Hasbullah, N.A. Azis, N. F. Daud, F. M. Rasad and M. M. Lassim. 2015. Morphogenesis studies in *Amaranthus gangeticus in vitro*. In Proc. International Conference on Agricultural, Ecological and Medical Science, Phuket, Thailand.

Arya, I. D., T. N. Chakravarty and S. K. Sopory. 1993. Development of secondary inflorescences and *in vitro* plantlets from inflorescence cultures of *Amaranthus paniculatus*. Plant Cell Reports 12: 286 – 288.

Bagga, S., K. Venkateswarlu and S. K. Sopory. 1987. *In vitro* regeneration of plants from hypocotyl segments of *Amaranthus paniculatus*. Plant Cell Reports 6: 183 – 184.

Bennici, A. and S. Schiff. 1997. Micropropagation of *Amaranthus*. High technology and micropropagation, pp. 20 -28. In: Bajaj YPS (ed.). Biotechnology in Agriculture and Forestry 39. Springer-Verlag, Berlin, Heidelberg, Germany.

Bennici, A., S. Schiff and R. Bovelli. 1992. *In vitro* culture of species and varieties of four *Amaranthus* L. species. Euphytica 62:181- 186.

Bennici, A., T. Grifoni, S. Schiff and R. Bovelli. 1997. Studies on callus growth and morphogenesis in several species and lines of *Amaranthus*. Plant Cell, Tissue and Organ Culture 49: 29 – 33.

Biswas, M., S. S. Das and S. Dey. 2013. Establishment of a stable *Amaranth tricolor* callus line for production of food colorant. Food Sci Biotechnology 22:1-8.

Coimbra, S. and R. Salema. 1994. *Amaranthus hypochondriacus*: Seed structure and localization of seed reserves. Ann. Bot. 74: 373-379.

Corke, H., Y. Cai and M. Sun. 2003. Antioxidant activity of betalains from plants of the Amaranthaceae. J. Agric. Food Chem. 51: 2288 – 2294.

Flores, H. F., A. Thier and A. W. Galston. 1982. *In vitro* culture of grain and vegetable amaranths (*Amaranthus* spp.). Amer J Bot 69: 1049-1054.

Guidea, S. D., N. Babeanu, O. Popa, D. Stanciu and I. Popa. 2012. Preliminary studies on *in vitro* behavior of various somatic explants from some cultivated *Amaranthus* genotypes. Scientific Bulletin, Series F, Biotechnologies 16: 9-14.

Leathers, R. R., C. Davin and J.P. Zryd. 1992. Betalain producing cell cultures of *Beta vulgaris* L. var. Bikores Monogerm (red beet). In Vitro Cell. Dev. 28: 29 – 45.

Mazza, G. and E. Miniati. 1993. Anthocyanin in fruits, vegetables and grains, pp. 1- 362. CRC Press Inc. Florida, U.S.A.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Physiol 15: 473 – 497.

Pant, K. C. 1983. Studies on the nutritional quality of grain amaranths. Nutr Rep Int 28: 1445 – 1457.

Salisbury F.B and C. W. Ross. 1992. Plant Physiology, 4th Ed. Wadsworth Publishing Company, Belmont, California. 682 p.

Singh, A., T. Kandasamy and B. Odhav. 2009. *In vitro* propagation of *Alternanthera sessilis* (sessile joyweed), a famine plant. African J of Biotech 8: 5691 – 5695.

Tisbe, V. O. and T. G. Cadiz. 1967. Amaranth or Kulitis. pp. 270-273. In: Knott JE and JF Deanon Jr. (eds.). Vegetable production in Southeast Asia. Philippines. University of the Philippines Los Baños, College, Laguna. 366 p.

Yaacob, J. S., L. C. Hwei, R. M. Taha, N. A. Mat and N. Aziz. 2012. Pigment analysis and tissue culture of *Amaranthus cruentus* L. ISHS Acta Horticulturae 958: 171-178.

Willis, J C. 1973. A dictionary of flowering plants and ferns. Cambridge University Press, Cambridge, UK

Wink, M. 2000. Functions of plant secondary metabolites and their exploitation in biotechnology. Annual Plant Reviews Vol. 3. CRC Press LLC, Florida, U.S.A. 410 p.