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Full Length Article

Digital Analysis on Cone-thorn Density and Morphology in Mutant *Aralia elata* Seedlings Induced by Ethyl Methanesulfonate

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Abstract

Natural *Aralia elata* reserves have been heavily exploited for medical and edible uses, resulting in an essential demand to rehabilitate the understory population using artificially cultured seedlings. Thorns around the cone tip generate a significant obstacle to affect the efficiency of *A. elata* development. In this study, ethyl methanesulfonate (EMS) was employed to induce mutagenesis and then growth and biomass in *A. elata* seedlings. EMS was applied to seedlings by root soaking or whole-plant spraying at doses of 0 (control), 19 (EMS-19), 38 (EMS-38) and 76 μM (EMS-76). Height growth was higher in the spraying treatment by 10% than in the root-soaking treatment. Compared to the control, the EMS-38 treatment resulted in lower cone-thorn density (1.3 individuals cm^{-2}) and the EMS-76 treatment resulted in lower fine root biomass (0.07 g plant^{-1}), length (215 cm), and tip-number (39.8). Through digital analysis on shoot image, EMS-treated seedlings had lower green-color index (41–56) than controlled seedlings (61–84). Color and brightness indices were positively correlated with fine root growth and biomass, while red-color index had a positive relationship with height growth. © 2019 Friends Science Publishers

Keywords: NWFP; Precise agriculture; Mutagenesis; Nursery culture; Breeding botany

Introduction

Araliaceae is a large family containing of 50 genera and 1412 species (Prasanth *et al.*, 2017). The genus *Aralia* out of Araliaceae has 71 species distributed across Asia and America continents (Arora *et al.*, 2015; Prasanth *et al.*, 2016). Many species from *Aralia* genus are taken as traditional medicine in Eurasian countries (Shikov *et al.*, 2016; Sun *et al.*, 2017). The strong driving of the usage of *Aralia* plants has resulted in the over-exploitation of their natural reserve. Intensive reduction of natural *Aralia* population had interrupted the ecological balance in their inhabited forest community. Therefore, with the aim to enable the sustainable development of understory *Aralia* populations, it was encouraged to raise an increasing number of *Aralia* seedlings in forested-areas even using the farmland as nursery. For example, since 2016 Chinese government established the Key Research and Development Program at national scale with the aim to promote understory non-wood forest product (NWFP) rehabilitation in the northeastern forests and *Aralia* plants are one of the main objectives in this big program (He and Yu, 2016). In the current literature, quite less has been documented about the approach to culture *Aralia* stocks than about either tree seedlings or agricultural crops. To efficiently produce quality seedlings has hindered the rehabilitation of *Aralia* population to a great extent.

A. elata is widely taken as a well-known homology of both medicine and wild-food in the Russian Far East, China, Korean, and Japanese (Shikov *et al.*, 2016; Sun *et al.*, 2017). Like other members in the Araliaceae family, natural population of *A. elata* has also been heavily explored and main products derived from this species are depending on artificially established population. Thorns around the cone-tip of main stem contribute to the significant recognition of *A. elata* in the full age-ranges. Cone-thorns also generate a critical restriction to the efficiency in thinning, harvest, and transplant of nursery-cultured *A. elata* seedlings. However, conventional cultural experience failed to bring available approach to cope with the issue of thorns' presence.

Naturally genetic variation can allow the preconditioning of breeding hybrid *A. elata* with the rare-thorn character, but the millions-of-years of evolution of

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Araliaceae family made it nearly impossible to seek out the rare-thorn from the natural population (Kim *et al.*, 2017). Instead, artificially induced mutation is an available approach to obtain the targeted character by broadening the genetic variation in a short term. The chemical mutagenesis with varied growth traits has been successfully introduced by ethyl methanesulfonate (EMS) used in several plant species (Lakhssassi *et al.*, 2017; Fischer *et al.*, 2018; Lee *et al.*, 2018). Due to the outcome of stable and efficient mutation to a broad range of genetic variation in morphological traits, the employment of EMS can be considered as a stimuli candidate to induce the decline of cone-thorn number in *A. elata* seedlings.

Remote sensing technique has been used in agriculture estimation for about three decades. Analyses on spectra from medium-resolution (e.g., 250 m × 250 m) satellite imageries are still accumulating (Abdi *et al.,* 2018; Adami *et al.,* 2018). Swift development of mobile termination has contributed to the prompt response of digital capture of image in high resolution. This motivated phytologists to employ digital analysis on target plants to fast measure their morphology and growth under some intentional conditions (Rabara *et al.*, 2017; Gupta *et al.,* 2019; Muller-Linow *et al.*, 2019; Zhu *et al.,* 2019). Digital analysis on plant surface can generate outcomes about not only growth size at the whole-plant scale (Gupta *et al.,* 2019) but also color and brightness at the specific-organ scale (Rabara *et al.,* 2017; Zhu *et al.*, 2019). These results facilitated the easy measurements that had physiological cues with inherent chlorophyll content, nutrient uptake, and antioxidant activity. Therefore, digital analysis promotes the possibility of precisely counting thorn number at the micro-scale in centimeter with high-resolution surface images.

In this study, *A. elata* seedlings were treated by EMS in several rates in two different ways so as to detect the potentially interactive effect of EMS and treating type on responsive mutagenesis hopefully with reduced number of cone-thorns. The simultaneously induced morphology and biomass changes were measured to detect their responses to the mutated varieties and their correlation with thorn number. It was hypothesized that (i) EMS treatment can induce the rare-thorn characteristic in *A. elata* seedlings in medium to high doses, (ii) wherein growth, biomass, and surface color and brightness can also be induced to change in mutagenesis, and (iii) cone-thorn density had a negative relationship with other variables in *A. elata* seedlings. The objective of this study was to test the possibility to induce a scare-thorn mutation of *A. elata* seedlings by the treatment of EMS and to detect the relationship between any couple of the morphological traits given by computer digital analysis.

Materials and Methods

Plant Material

A. elata seeds were collected from a natural population (44°53' N, 129°16' E) in Mudanjiang, Heilongjiang Province, Northeast China. All seeds were collected from uniform size of mature shrubs. Collected seeds were sterilized using potassium hypermanganate at the rate of 0.5% and stored in sands for a year in Lushuihe, Fusong, Jilin, China. In April 2017, seeds were excavated out, sterilized again, soaked in water for 12 h. Germinated seedlings were raised for a growing season in 2017. In April 20th, 2018, ten thousand one-year-old *A. elata* seedlings werescreened for uniform-sized ones in a local nursery (Zhilunpudao A&F Sci. Co., Changchun, Jilin, China). In April 27th, 2018, eight thousand seedlings passed the screening and they were chosen of a uniform size and transplanted to the experimental nursery (45°44' N, 126°43' E), college of Horticulture and Landscape, Northeast Agricultural University, Harbin, China. All seedlings were firstly treated by cutting fine roots to retain 40% of initial length and divided into two groups with 4,000 individuals for each group.

Chemical Treatment

Seedlings from one group were treated with EMS (Sigma-Aldrich, Chaoyang District, Beijing, China) aqueous solutions by root soaking and those from the other group were treated by whole-plant spraying. Both types of treatments employed EMS solutions at one of the four concentrations (v/v) of 0 (control), 19 (EMS-19), 38 (EMS-38), and 76 μM (EMS-76) (Nascimento *et al.*, 2015; Lakhssassi *et al.,* 2017; Lee *et al.*, 2018). Seedlings from the root-treated group were sprayed by distilled water, bagged by plastic package to the whole shoot, and soaked in EMS solutions. Meanwhile the whole-plant-treated group were sprayed by EMS solution, shoot-bagged, and soaked in distilled water.

We employed a random block design with each of the four treatments, including the control, being arranged as one of the 10 replicated blocks. All blocks were randomly placed to eliminate the possible edge effect. Within each block, 100 seedlings were randomly planted to the commercial substrate with peat and spent mushroom residue (Zhiluntuowei A&F Sci. Co., Changchun, Jilin, China) in plastic pots (top diameter × bottom diameter × height, 25 cm × 17 cm × 18 cm). Planted seedlings were cultured for six months including five-month greenhouse growing and one-month hardening defoliation.

Sampling and Measurements

In mid-October, 2018, when all seedlings defoliated 30 seedlings were randomly sampled from one combined treatment block. Sampled seedlings were firstly measured for height and diameter 1 cm above the root-collar (RCD), and divided into above-ground (shoot) and below-ground parts (root). One shoot part was placed on the black-background experimental table and photographed with the camera in Apple iPhone VIs (5–8 million pixels, Apple Inc., Infinite Loop Cupertino, CA, USA) 40 cm above the table.

On the other hand, fine roots (<1 mm in diameter) were excised and scanned to generate a projected image in the resolution of 118.11 pixels/cm (HP Deskjet 1510 scanner, HP Inc., Palo Alto, CA, USA). Thereafter, shoots, coarse roots, and fine roots were all oven-dried at 70°C for 72 h and measured for biomass. Shoot photos were treated by Photoshop (ver. 8.0, Adobe® Systems Incorporated Inc., San Jose, California, USA) to eliminate all background colors and analyzed for cone-thorn density and the scores for red, green, and blue color-indices, total color index, and brightness. Fine root morphology was analyzed by WinRhizo (Regent Instruments Inc., Canada).

Data Analysis

SAS software (ver. 9.4 64-bit, SAS Institute Inc., NC, USA) was used to analyze the effects. Combined effects of four treatments and two treated organs were tested by analysis of variance (ANOVA). When significant effect was indicated, results were compared by Tukey test at 0.05 level. The Pearson correlation was analyzed for couples of any two of parameter data.

Results

Growth and Biomass

The difference of treated-organs had a significant effect on plant height growth, which was lower in the treatment to the roots (2.40±0.38 cm) than to whole-plant (2.67±0.61 cm) by 10%. The EMS treatment had a significant effect on cone-thorn density (Fig. 1) and fine root biomass and morphology (Table 2). Compared to the control, the EMS-38 treatment decreased the cone-thorn density by 46% and the EMS-76 treatment resulted in the lower fine root biomass, length, and tip numbers by 30%, 54%, and 39%, respectively (Table 2).

Digital Analysis on Scanned Shoot Part

The EMS treatment and effective organs had an interactive effect on all scanned indices in the shoot part (Table 1). Generally, seedlings exposed to the EMS-19 and EMS-38 treatments both in root and whole-plant had lower scanned indices than controlled seedlings treated in the whole-plant (Table 3). The EMS-76 treatment resulted in higher red-color index by 28% relative to the controlled plants to the root organ.

Correlation Analysis

Seedling height had positive correlation with both red- and color-indices (Table 4). Fine root biomass had positive correlation with all scanned indices and fine root morphologies. Cone-thorn number had no relationship with other scanned indices, which all positively to each other.

Fig. 1: Typical performances of cone-thorn density in mutant *Aralia elata* seedlings treated by ethyl methanesulfonate (EMS) at doses of 0 (Control), 19 (EMS-19), 38 (EMS-38), and 76 μM (EMS-76)

Discussion

Our results showed that EMS failed to induce mutagenesis with any changes in morphology growth and biomass accumulation in shoot part of *A. elata* seedlings. We did not find any significant effect of EMS on height growth, which concurred with the results found on capsicum (Nascimento *et al.*, 2015). In contrast, our results were contradicted to those found in some mutant lines of lupin (Fischer *et al.*, 2018) and wheat (Lu *et al.*, 2015). We found that both our seedlings and those studied by Nascimento *et al.* (2015) were measured for shoot morphology directly after receiving EMS, but mutagenesis with altered shoot growth was found on F_2 generation. Maybe our treatment using EMS on *A. elata* was not long enough to induce genetic mutation.

Instead of EMS dose, the difference of treated organ had a significant effect on height growth. These results suggest that the efficiency of increasing height through genome modification by exogenous EMS was higher through permeating the shoot cells than allocation through root soaking. To our knowledge we failed to find any studies that employed similar EMS treatments to different plant organs. It was summarized that EMS was ever used to treat plants by three main accesses, which were isolated microspores, haploid embryos, and haploid embryogenesis from mutant donor plants (Prem *et al.*, 2012). We surmise that the EMS treatment to the shoot part by spraying can induce DNA polymerases to catalyze the thymine instead of cytosine during DNA replication in a higher frequency than the treatment to roots, which may probably affect DNA replication after mobilizing EMS upwards to the shoot part.

The fine root results at least partly supported our second hypothesis by decreased biomass in the EMS-76 treatment. This response was accompanied by changes in root morphology of length and tip number, clearing indicated that the decline of fine root biomass was caused by restricted fine root come-out and elongation. These data suggested that EMS treatment at 76 μM tended to induce negative influence on the quality of *A. elata* seedlings. Our results contradicted to those in King *et al*. (1995),

Table 1: *P* values from ANOVA analysis of ethyl-methanesulfonate treatment, treated organ, and their interaction on growth, morphology, and biomass parameters in mutant *Aralia elata* seedlings

Parameter	Treatment	Organ	Treatment × Organ
Height	0.0984	0.0451*	0.4715
Root-collar diameter	0.3096	0.3475	0.2478
Shoot biomass	0.2512	0.0831	0.4952
Fine root biomass	0.0310*	0.0594	0.9031
Coarse root biomass	0.5057	0.6837	0.0823
Cone-thorn density	0.0007**	0.8347	0.5906
Red-color index	<0.0001***	<0.0001***	0.0366*
Green-color index	<0.0001***	<0.0001***	**0.0383***
Blue-color index	<0.0001***	<0.0001***	**0.0449***
Color index	<0.0001***	<0.0001***	**0.0361***
Brightness	<0.0001***	<0.0001***	**0.0357***
Root length	<0.0001***	0.7992	0.9782
Root-tip number	**0.0094****	0.2450	0.5311

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

Table 2: Growth, cone-thorn density, and root morphology in mutant *Aralia elata* seedlings treated by ethyl-methanesulfonate (EMS) at doses of 19 (EMS-19), 38 (EMS-38), and 76 µM(EMS-76)

Parameter	Control	EMS-19	EMS-38	EMS-76
Shoot biomass (g)	0.19±0.07	0.18±0.06	0.22±0.10	0.16±0.07
Coarse root biomass (g)	0.68±0.03	0.86±0.04	0.81±0.08	0.76±0.03
Fine root biomass (g)	0.10±0.03a[1]	0.09±0.03ab	0.09±0.04ab	0.07±0.03b
Cone-thorn density (individuals/cm^2)	2.40±0.71a	1.90±0.56ab	1.30±0.70b	1.75±0.73ab
Root length (cm)	466±107a	316±132bc	353±147ab	215±61c
Root-tip number	65.3±29.79a	45.0±17.74ab	47.3±18.98ab	39.8±14.00b

[1] Different letters in a row indicate significant difference at 0.05 level

Table 3: Scanned results for shot morphology in mutant *Aralia elata* seedlings treated by ethyl-methanesulfonate (EMS) at doses of 19 (EMS-19), 38 (EMS-38), and 76 µM (EMS-76) in root and whole-plant organs

Treatment	Organ	Red-color index	Green-color index	Blue-color index	Color index	Brightness
Control	Root	84.61±29.51c[1]	61.37±23.72abc	40.01±16.57abc	61.99±23.24bc	65.90±24.63bc
Control	Whole	114.36±19.11a	84.34±16.71a	56.02±12.62a	84.90±16.00a	90.12±16.88a
EMS-19	Root	67.10±10.76c	47.85±8.94c	30.56±6.66c	48.47±8.72c	51.65±9.18c
EMS-19	Whole	78.52±8.68c	56.02±7.01bc	36.15±5.14bc	56.90±6.89c	60.40±7.22c
EMS-38	Root	58.25±18.40c	40.51±13.47c	25.29±8.50c	41.35±13.41c	44.09±14.36c
EMS-38	Whole	65.59±12.48c	46.44±8.96c	30.10±5.69c	47.28±8.94c	50.28±9.60c
EMS-76	Root	66.54±15.80c	46.33±11.90c	29.01±8.43c	47.30±12.03c	50.41±12.67c
EMS-76	Whole	108.29±22.68ab	79.09±19.04ab	52.28±14.23ab	79.89±18.60ab	84.80±19.56ab

[1] Different letters in a column indicate significant difference at 0.05 level

where a nuclear EMS mutant of Arabidopsis was found to display extreme proliferation of roots. However, the root growth response to EMS treatment highly varied depending on combination between addition dose and treatment time. For example, an effort on Stevia (*Stevia rebaudiana* Bertoni) reported a range of responses of root biomass to EMS treatments and both proliferated and depressed root growth appeared meanwhile (Gerami *et al.,* 2017). In addition, experiments with various responses of root growth after the EMS treatment were raised under some given conditions. Singh *et al.* (2014) found reported an unchanged root growth in EMS-treated Arabidopsis mutant but plantlets therein were placed under low phosphate conditions. In another study, Hermans *et al.* (2010) found a similar response of root growth between EMS-mutagenized and wild-type Arabidopsis plants when grown on low to moderate nitrate concentrations, but EMS-mutants had depressed root elongation and less number of lateral roots in high-nitrate stress. Therefore, more possible responses of root biomass and morphology were likely to be detected in *A. elata* seedlings exposed to EMS in a wider range of doses and treatment time.

Reduced density of cone-thorn in the EMS-38 treatment compared to the control supports our first hypothesis. To our knowledge it is the first time for us to report the rare-thorn trait in EMS-treated *A. elata* mutants hence it is quite difficult to find similar results as reference. We noticed that in a higher dose of EMS treatment at 76 µM, where root growth exhibited a depressive response compared to the control, cone-thorn had no difference with that of control. This suggested that the underlying difference of EMS doses for mutants with changed thorn density and root growth. Accordingly, we did not find any relationship between cone-thorn density and any other variables, suggesting that the mutational trait of less thorn density was irrelevant to any other changes. Swaroop *et al.* (2015) induced Bougainvillea mutants by exposing to gamma rays but they failed to obtain significant difference of thorn density per branch in response to a range of gamma ray doses. Future work is suggested to figure out the mechanism for the change of thorns in *A. elata* mutants by EMS.

Table 4: Pearson correlations between couples of parameters about growth parameters and scanned indices

Variables		Height	RCD	ShootBio^{1}	Froot^{2}	Croot^{3}	Thorn^{4}	R^{5}	G^{6}	B^{7}	Bright	Color^{8}	Rlength^{9}
RCD	*R*	0.032											
	P	0.940											
ShootBio	*R*	0.594	0.648										
	P	0.120	0.082										
Froot	*R*	0.565	0.690	0.690									
	P	0.144	0.058	0.058									
Croot	*R*	0.270	-0.107	0.268	0.157								
	P	0.518	0.801	0.521	0.711								
Thorn	*R*	-0.415	0.478	-0.233	0.321	-0.277							
	P	0.306	0.231	0.579	0.438	0.506							
R	*R*	0.716	0.459	0.590	0.929^{11}	0.151	0.268						
	P	0.046	0.253	0.124	0.001	0.721	0.520						
G	*R*	0.706	0.457	0.574	0.922	0.144	0.287	1.000					
	P	0.050	0.255	0.137	0.001	0.733	0.491	<0.001					
B	*R*	0.704	0.454	0.563	0.918	0.134	0.297	0.999	1.000				
	P	0.052	0.258	0.146	0.001	0.752	0.475	<0.001	<0.001				
Bright	*R*	0.709	0.457	0.578	0.922	0.146	0.281	1.000	1.000	1.000			
	P	0.049	0.255	0.133	0.001	0.730	0.500	<0.001	<0.001	<0.001			
Color	*R*	0.709	0.457	0.579	0.922	0.146	0.281	1.000	1.000	1.000	1.000		
	P	0.049	0.255	0.133	0.001	0.731	0.500	<0.001	<0.001	<0.001	<0.001		
Rlength	*R*	0.249	0.587	0.378	0.818	-0.168	0.477	0.752	0.754	0.747	0.753	0.752	
	P	0.551	0.126	0.356	0.013	0.690	0.232	0.031	0.031	0.033	0.031	0.031	
Tips	*R*	0.353	0.493	0.329	0.830	0.069	0.603	0.887	0.894	0.893	0.892	0.891	0.860
	P	0.391	0.215	0.426	0.011	0.870	0.114	0.003	0.003	0.003	0.003	0.003	0.006

^{1}ShootBio, shoot biomass; ^{2}Froot, fine root biomass; ^{3} Croot, coarse root biomass; ^{4}Thorn, cone-thorn density; ^{5}R, red-light index; ^{6}G, green-light index; ^{7}B, blue-light index; ^{8}Color, color index; ^{9}Rlength, root length; ^{10}Tips, root tip number; ^{11} Values in bold font indicate significant correlation

The color in visible lights on plants depends on how they are structured and pigmented (van der Kooi *et al.*, 2014). Red (R) light mainly falls in the wavelength of 600-700 nm which was indicated to be able to suggest biomass and nitrogen (N) uptake in field rice plants (Huang *et al.*, 2017). The blue (B; 400-500 nm) light was found to be closely related to chlorophyll content in leaves (Kawashima and Nakatani 1998). Green (G; 500-600 nm) light color index given by Photoshop-software has been found to be negatively correlated with foliar N concentration in pepper (Zhu *et al.*, 2019). In our study, all light indices were analyzed by reflection from defoliated rootstock of *A. elata*, where all light indices tended to be lower in the EMS-induced mutants than in untreated control. Because no G leaves were attached to the stem, our results about color indices unlikely to be the result of photosynthetic organs hence the variation of coloration were mainly derived from the variation of structure and reserve materials (van der Kooi *et al.,* 2014). Correlation analysis indicated that our color indices were related to root biomass and growth, which concurred with those by Huang *et al.* (2017). In addition, according to Zhu *et al.* (2019), color difference may also result from the difference of reserved N. In addition, both R- and color-indices had positive relationship with height growth. This was because R light dominates the visible light by higher wavelength than G and B lights. R light was also effective to promote woody plant height growth (Li *et al.,* 2018).

Conclusion

A dose of 76 μM EMS treatment resulted in decreased fine root biomass and root morphologies of length and tip number. The medium dose of 38 μM EMS treatment reduced the cone-thorn density, which, however, had no relationship with any other variables. Instead, shoot color indices were generally lower in EMS-mutants than the control and had a positive relationship with root biomass and morphology. Therefore, the employment of EMS in the dose of 38 μM can be used to induce *A. elata* mutants with the low-thorn-density trait, which could be captured by digital camera and analyzed by software. EMS-mutants tended to have lower scores of surface color indices, which suggested the sign of depressed root growth.

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