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QL conducted the whole study; WJ and YR prepared the root tip samples; RC and XL detected the contents of bioactive compounds; YY and HW suggested the idea and prepared the manuscript, they had equal contributions to the work

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ORIGINAL RESEARCH PAPER

In vitro cloning potential and phytochemical evaluations of aneuploid individuals produced from reciprocal crosses between diploid and triploid in *Echinacea purpurea* L.

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Abstract
Aneuploidy often presents large variations in morphology, physiology, biochemistry, and genetics owing to karyotypic imbalance. This study aimed to evaluate the efficacy of aneuploid breeding in *Echinacea purpurea* L, an important medicinal plant. Reciprocal crosses between diploid and triploid plants were performed to generate aneuploid plants. Cross with triploid as female parent resulted in increased production of aneuploid individuals (19 of 23; 82.61%), while using diploid as female parent yielded much higher percentage of diploid progenies (130 of 133; 97.74%). Each aneuploid had particular karyotypic characteristics compared to the parents. The proportions of median, submedian, and subterminal centromere location chromosomes in gross chromosomes among aneuploids and two parents showed large variations. Although aneuploids had relatively lower adventitious bud regeneration rates than their parents, almost half of them looked morphologically normal, with high survival rates when transplanted to ex vitro conditions. Among the bioactive compounds assessed, cichoric acid and chlorogenic acid contents were extremely encouraging. Most aneuploids had higher cichoric acid and chlorogenic acid contents than their parents. For example, A2 had the highest cichoric acid content of 21.98 mg/g dry weight, more than twice the values of diploid and triploid. Meanwhile, A21 had the highest chlorogenic acid content of 1.84 mg/g, approximately five times more than the parental values. Eleven superior aneuploid lines were successfully screened as breeding candidates. The present findings indicated *E. purpurea* is highly tolerant of karyotypic imbalance and aneuploid plants could serve as prospective breeding resources in *E. purpurea*.

Keywords
reciprocal cross; adventitious bud regeneration rate; morphology of in vitro plantlet; cichoric acid; chlorogenic acid

Introduction

In the breeding field, ploidy manipulation is a valuable tool for crop quality improvement; and polyploidy, especially tetraploidy induction has been adopted as an efficient breeding strategy [1,2]. Failure of chromosomes or chromatids to separate properly to opposite poles during meiosis or mitosis in above polyploidy often results in the occurrence of

aneuploidy. Aneuploidy involves gain or loss of individual chromosome(s) or chromosome segment(s). Therefore, gene dosage balance in aneuploidy is disrupted and may bring about events such as chromosomal rearrangements, DNA sequence changes, and gene expression changes [3,4]. These abnormalities lead to multiple variations in plants, including morphology, physiology, biochemistry, and genetics [5]. A portion of aneuploids grow healthily and serve as cultivated resources, as demonstrated for *Betula humilis* [6] and many garden chrysanthemum cultivars [7].

Echinacea purpurea L. ($2n = 2x = 22$), an important herbaceous plant indigenous to North America, is well known for its anti-inflammatory and immunomodulatory properties [8,9]. *Echinacea purpurea* is widely used for pharmaceutical preparations in many countries in Europe, North America, and Australia [10]. Alkamides, caffeic acid derivatives and polysaccharides are the major bioactive compounds in *E. purpurea* [11,12]. Cichoric acid, the most abundant caffeic acid derivative in *E. purpurea*, is considered one of the most potent HIV-1 integrase inhibitors [13]. Indeed, the quality of the herb is usually assessed by cichoric acid levels [14,15]. Chlorogenic acid, a natural polyphenol product, possesses diverse biological properties such as antibacterial [16], antiviral [17], and hepatoprotective [18]. Meanwhile, *E. purpurea* also attracts considerable attention for its ornamental value. It is widely cultivated as ornamental plant and for cut-flower production. In recent years, breeding of *E. purpurea* mainly focused on the ornamental value [19], and developing new medicinal cultivars with high bioactive compounds was neglected. Nowadays, naturally occurring *E. purpurea* populations are largely exhausted by wild crafting. Commercial cultivation of *E. purpurea* used medicinally is considered an alternative method to meet the increasing market demand [20]. Thus, improving the contents of bioactive compounds becomes the general trend in the field. We have recently made some progress on polyploidy induction, including tetraploid [21], triploid [22], and octoploid [23] organisms. Polyploidy presented higher bioactive compounds contents than diploidy [24,25]. Now, as the karyotypic particularity of aneuploidy, developing new aneuploid lines with high contents of functional compounds may be innovative and promising. There are currently no reports regarding *E. purpurea* aneuploidy breeding.

Aneuploidy usually exists in the progenies of interploidal hybridization that involved polyploid as male or female parent [5,26]. It has been demonstrated that interploidal hybridization is important for generating variant and viable progenies, expanding population diversity, and promoting gene exchanges between parents [27–30]. In this work, reciprocal crosses among diploid, triploid, and tetraploid plants were performed to generate aneuploid progenies. Then, the main characteristics were analyzed that affected the evaluation of *E. purpurea* as a cultivar, i.e., in vitro cloning potential (including adventitious bud regeneration rate and in vitro plantlet morphology) and the contents of the main bioactive compounds. The main objectives of our study were to evaluate *E. purpurea* tolerance to karyotypic imbalance and produce new superior (especially highly-producing secondary metabolites) aneuploids to satisfy the market demand for *E. purpurea* products.

Material and methods

Plant material

Among the six diploid lines described in Li et al. [31], genotype F that showed the highest cloning potential was selected as original diploid material, for tetraploid induction via in vitro colchicine treatment [21] and crossing experiments. Triploid plants used for crossing were produced from the crossing of the above-mentioned diploid and tetraploid plants.

Controlled pollination

Diploid, triploid, and tetraploid plants were grown in flowerpots under routine care. As *E. purpurea* has cross-pollination and self-incompatibility features [32,33], more

Fig. 1 The inflorescence structure of *Echinacea purpurea* L.

than 10 inflorescences per plant were bagged several days before blooming to prevent outcrossing. Reciprocal crosses were carried out between diploid and triploid plants as well as triploid and tetraploid plants. In each cross combination, 1500 florets in 10 inflorescences of three plants were pollinated. Pollens were collected from the bagged inflorescences at the full bloom stage, and immediately used for crossing or stored at 4°C. Pollination was performed by directly placing the fresh or stored pollens onto the stigmas of the female parent. Fig. 1 shows the inflorescence structure of *E. purpurea*. The pollinated inflorescences were bagged to exclude random pollination.

Progeny seed germination and ploidy state analysis

Seeds were harvested from the infructescences of all crossed female parents at maturity. They were placed on filter paper soaked with 3 mg/L gibberellic acid aqueous solution overnight, and surface-sterilized by immersion in 70% (v/v) ethanol for 30 s and 1% sodium hypochlorite (in water) for 10 min. This was followed by three rinses in sterile distilled water. Then, all the disinfected seeds were sown on Murashige and Skoog (MS) medium [34] without hormones. The germination culture was kept in the dark for the first week and then incubated under light conditions at 25 ±2°C. Each germinated progeny seedling was assigned a code. When the seedling roots elongated to about 20 mm, the ploidy state was confirmed according to the chromosome observation method as described previously [21]. Actively growing root tips were collected from each seedling, pretreated with 0.05% colchicine for 5 h, and fixed in ethanol / acetic acid (3:1) for 20 h at 4°C. The fixed root tips were then washed three times in distilled water and hydrolyzed in 1 N HCl at 60°C for 8 min, after which the root tips were rinsed three times with distilled water again. Then, the root tips were placed on a microscope slide and stained with a drop of carbolfuchsin. The preparation was gently squashed beneath the coverslip, and chromosomes in metaphase spreads were counted under a light microscope.

Karyotypic analysis of aneuploid progeny seedlings

The karyotypic features were assessed in at least 10 well-spread metaphase plates from each seedling. The chromosomes were photographed at ×1000 magnification on an Axio Observer A1 microcope (Zeiss, Germany). The length of the chromosomes, including long and short arms, was measured with Adobe Photoshop CS5 (Adobe Systems, Inc., USA). The same software was used for chromosome arrangement. The classification of chromosomes was performed according to the arm ratio (r = length of long arm / length of short arm) as described by Levan et al. [35].

Adventitious bud regeneration and rooting culture

Leaf, petiole, and root explants (leaf explants, about 0.6 cm^2 in area; petiole and root explants, about 0.8 cm in length) of each progeny seedling were dissected and inoculated on MS regeneration medium supplemented with 0.4 mg/L 6-benzyladenine (BA) and 0.01 mg/L naphthaleneacetic acid (NAA) for adventitious bud regeneration. Each treatment included six replicates with consisting of five explants per replicate. Forty-five days later, the number of adventitious buds regenerated from the explants of each seedling was counted to determine the adventitious bud regeneration rate and evaluate the in vitro cloning potential of the progeny seedlings. Adventitious bud regeneration rate = No. of regenerated buds / No. of explants. Then, actively growing buds regenerated from all three explant types were isolated and inoculated on MS rooting medium supplemented with 0.015 mg/L NAA for rooting. Forty-five days later, the most representative in vitro plantlet of each aneuploid progeny was photographed.

Medium preparation and culture conditions

All media contained MS basal medium elements, 3% sucrose, and 0.7% agar, and were adjusted to pH 5.8 prior to autoclaving at 104 kPa at 121°C for 15 min. All cultures (except dark culture) in this study were maintained under controlled light condition with a 16-h photoperiod under cool-white fluorescent lamps (approximately 50 μmol m^{-2} s^{-1}) in a growth chamber with 25 ±2°C.

Determination of the main bioactive compounds

The aboveground and underground parts of 2-month-old plantlets in each progeny were collected and dried with hot air at 65°C for 72 h and ground to fine powder using porcelain mortars. After sieving the powder using a 200 mesh sieve, 0.1 g sample was extracted for 30 min in 10 mL of 70% ethanol by ultrasonication (40 kHz). The obtained solution was centrifuged for 5 min at 4000 rpm (Eppendorf 5804R, Germany). The resulting supernatant was collected and diluted with 70% ethanol to 10 mL, and filtered through a 0.45-μm microporous membrane. The filtrate was used for the assessment of bioactive compounds. Reference standards of cichoric acid, chlorogenic acid, echinacoside, caftaric acid, and 1,3-dicaffeoylquinic acid were dissolved in appropriate volumes of 70% ethanol, and diluted to 0.02, 0.04, 0.06, 0.08, and 0.1 mg/mL.

The amounts of bioactive compounds were determined by high-performance liquid chromatography (HPLC). Each sample was analyzed in triplicate. Equal amounts (10 μL) of each replicate were automatically injected into the HPLC system Agilent 1260 Infinity (Agilent Technologies, USA) equipped with an Inertsil/WondaSil C18 column (250 × 4.6 mm, pore size 5 μm; Shimadzu, Japan). The mobile phase was 1.80% (v/v) phosphoric acid (A) and acetonitrile (B), run at a flow rate of 1 mL/min for 22 min; the UV detector G1314F was set at 330 nm. The chromatograph was programmed as follows: 0–13 min, 90–78% A, 10–22% B, linear gradient; 13–14 min, 78–60% A, 22–40% B, linear gradient; 14–17 min, 60% A, 40% B, isocratic; 17–17.5 min, 60–90% A, 40–10% B, linear gradient; 17.5–22 min, 90% A, 10% B, isocratic. All standards were purchased from Sigma, USA. The concentrations were measured on a dry weight basis (DW, mg/g).

Statistical analysis

Data were analyzed statistically using the SPSS 19.0 software. Significant differences were determined using Duncan's multiple range test; $p < 0.05$ was considered statistically significant.

Results

Ploidy state of progeny seedlings obtained from crosses between diploid and triploid

Reciprocal crosses between triploid and tetraploid did not set seed. The numbers of seeds in reciprocal crosses between diploid and triploid were markedly different (Tab. 1). The number of seeds obtained from the cross using diploid as female parent was 143, while 36 seeds were obtained using triploid plant as female parent. Except for seeds that did not germinate, using triploid as the female parent resulted in increased production of aneuploid progeny (19 of 23; 82.61%) while using diploid as the female parent yielded much higher percentage of diploids (130 of 133; 97.74%). Among the 156 progeny seedlings obtained in reciprocal crosses, 21 aneuploid seedlings had continuous chromosome numbers, ranging from 23 to 31. Polyploid seedlings (e.g., triploid, tetraploid, and pentaploid) were found in both reciprocal crosses.

Tab. 1 Chromosome number distribution of progeny seedlings from crosses between diploid and triploid in *Echinacea purpurea* L.

Male parent	Female parent	No. of pollinated florets	No. of seeds set	No. of progeny seedlings	Ploidy state	Chromosome number	No. of progeny seedlings
Diploid	Triploid	1500	36	23	Euploid	22	1
						33	1
						44	1
						55	1
					Aneuploid	25	3
						26	5
						27	4
						28	1
						29	2
						30	2
						31	2
Triploid	Diploid	1500	143	133	Euploid	22	130
						44	1
					Aneuploid	23	1
						24	1

Karyotypic analysis of aneuploid seedlings from crosses between diploid and triploid

The concrete karyotypic characteristics of aneuploid progeny seedlings obtained from the above crosses are presented in Tab. 2. Diploid and triploid parents had similar karyotype, whose formulas were 22 = 8 median (m) + 4 submedian (sm) + 10 subterminal (st) and 33 = 12m + 6sm + 15st, respectively. The proportions of m, sm, and st in gross chromosomes in diploid and triploid were identical; and the proportions of them were 36.36%, 18.18%, and 45.45%, respectively. Compared with parents, each aneuploid had particular karyotypic characteristics. Some aneuploids had identical chromosome numbers, but different chromosome constitutions. For example, chromosome numbers of A3, A4, and A5 were 25, but their chromosome constitutions were 25 = 11m + 4sm + 10st, 25 = 6m + 7sm + 12st, and 25 = 8m + 4sm + 13st, respectively. Similar examples were found in other aneuploids (Tab. 2). Each aneuploid simultaneously consisted of three types of chromosomes, including m, sm, and st (Fig. 2, Tab. 2, Fig. S1). However, the proportions of m, sm, and st in gross chromosomes among all aneuploid progenies and their parents showed marked differences. Take the proportions of m, for example, diploid and triploid parents had an identical proportion of m (36.36%). Among the 21 aneuploids, six (e.g., A1 and A2) had similar proportions of m to the parents. The proportions of m in other 15 aneuploids (e.g., A3 and A4) were much different from those of the two parents. The proportions of sm and st were similar among the aneuploid progenies and their parents. Additionally, two chromosomes (Fig. 2, A3 and A18) were significantly shorter than others, which suggests that the structural chromosome changes occurred.

Tab. 2 Karyotypic characteristics of aneuploid progeny seedlings from crosses between diploid and triploid in *Echinacea purpurea* L.

Code*	Karyotypic formula	Proportion (%)		
		m	sm	st
D	22 = 8m + 4sm + 10st	36.36	18.18	45.45
T	33 = 12m + 6sm + 15st	36.36	18.18	45.45
A1	23 = 8m + 4sm + 11st	34.78	17.39	47.83
A2	24 = 8m + 4sm + 12st	33.33	16.67	50.00
A3	25 = 11m + 4sm + 10st	44.00	16.00	40.00
A4	25 = 6m + 7sm + 12st	24.00	28.00	48.00
A5	25 = 8m + 4sm + 13st	32.00	16.00	52.00
A6	26 = 6m + 7sm + 13st	23.08	26.92	50.00
A7	26 = 7m + 8sm + 11st	26.92	30.77	42.31
A8	26 = 8m + 7sm + 11st	30.77	26.92	42.31
A9	26 = 8m + 4sm + 14st	30.77	15.38	53.85
A10	26 = 9m + 4sm + 13st	34.62	15.38	50.00
A11	27 = 7m + 5sm + 15st	25.93	18.52	55.56
A12	27 = 9m + 10sm + 8st	33.33	37.04	29.63
A13	27 =8m + 6sm + 13st	29.63	22.22	48.15
A14	27 = 9m + 6sm + 12st	33.33	22.22	44.44
A15	28 = 9m + 6sm + 13st	32.14	21.43	46.43
A16	29 = 10m + 4sm + 15st	34.48	13.79	51.72
A17	29 = 9m + 6sm + 14st	31.03	20.69	48.27
A18	30 = 8m + 4sm + 18st	26.67	13.33	60.00
A19	30 = 8m + 6sm + 16st	26.67	20.00	53.33
A20	31 = 10m + 5sm + 16st	32.26	16.13	51.61
A21	31 = 11m + 6sm + 14st	35.48	19.35	45.16

* D – diploid; T – triploid; A1 to A21 – aneuploid.

Comparison of adventitious bud regeneration rates among aneuploids and their diploid and triploid parents

As shown in Tab. 3, the sums of adventitious bud regeneration rates of three explant types among aneuploid progenies and the parents were different. An aneuploid-A9 (6.81) showed a higher regeneration rate than the triploid parent (4.8), and comparable to the diploid parent (7.47). Meanwhile, four aneuploids-A21 (4.14), A8 (3.95), A16 (3.77), and A4 (3.6) had regeneration rates close to that of the triploid parent (4.8). Leaf explants regenerated the most buds in both diploid and triploid. Among the 21 aneuploids, except for A17 which did not regenerate any bud for the three types of explants, 12 aneuploids (e.g., A4 and A5) had the highest numbers of buds regenerated from leaf explants; three aneuploids (A1, A7, and A9) had the highest numbers of buds regenerated from petiole explants, while the remaining five (A2, A3, A8, A15, and A20) had the highest numbers of buds regenerated from root explants. For leaf explants, an aneuploid-A9 (2.74) showed comparable regeneration rate to the diploid (2.91) and

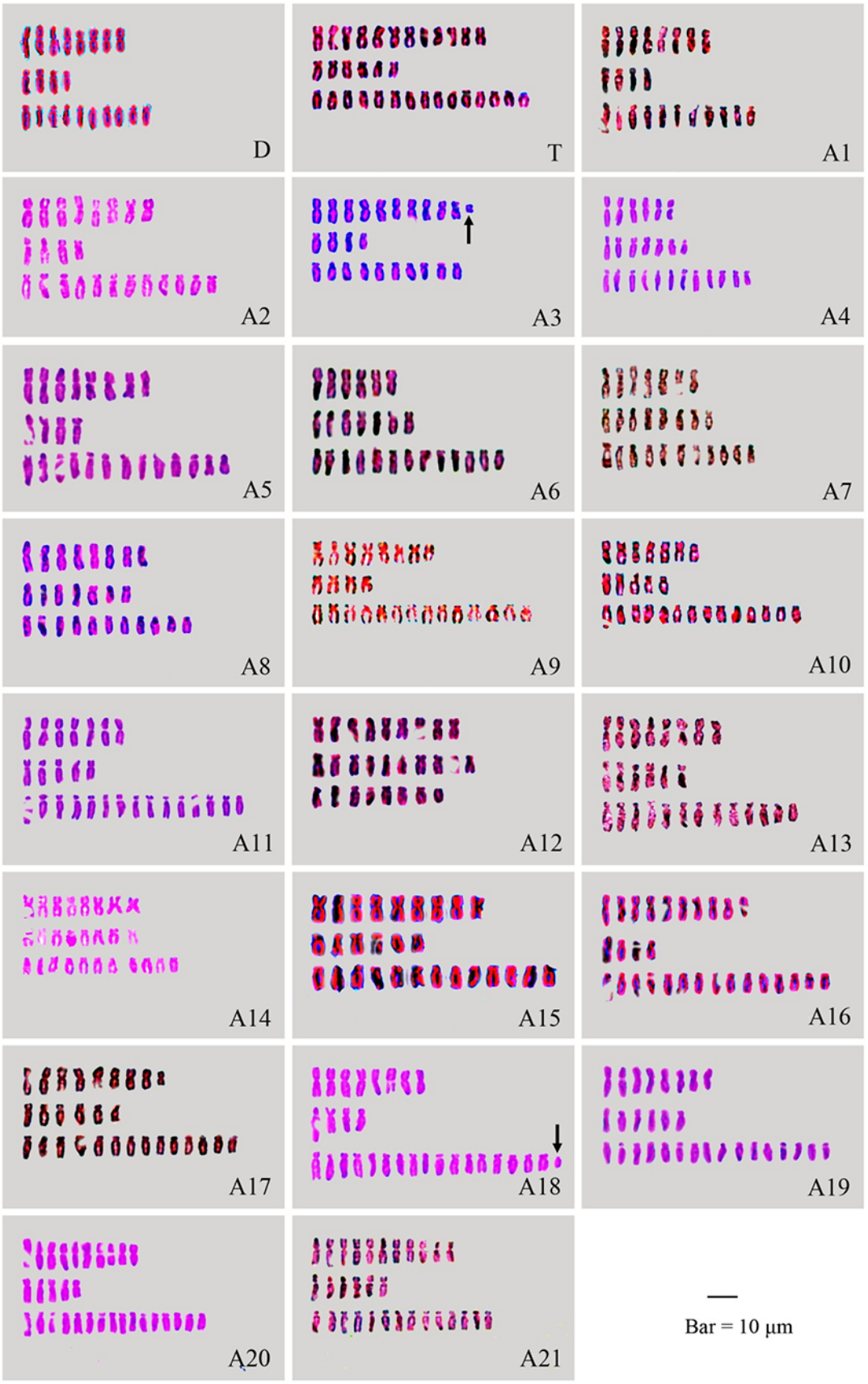

Fig. 2 Karyotypes of aneuploid progeny seedlings and their diploid and triploid parents in *Echinacea purpurea* L. For each photo, rows from top to bottom are m, sm, st, respectively. Chromosomes for each type are arranged according to the total length from long to short. If the total length of two chromosomes is the same, then the chromosomes are arranged according to the length of short arm from long to short. Codes in the photographs are consistent with those in Tab. 2. The arrowheads in A3 and A18 point to structurally changed chromosomes.

Tab. 3 Comparison of adventitious bud regeneration rates among aneuploid progenies and their diploid and triploid parents in *Echinacea purpurea* L.

Code*	No. buds regenerated/explant			Sum	Ranking
	leaf	petiole	root		
D	2.91 ±0.39 ^{Aa**}	2.87 ±0.48 ^{Aa}	1.69 ±0.47 ^{Ba}	7.47	1
T	2.85 ±0.87 ^{Aab}	1.25 ±0.37 ^{Bb}	0.70 ±0.17 ^{Cbcd}	4.80	3
A1	0.04 ±0.04 ^{Bde}	0.21 ±0.21 ^{Ab}	0.05 ±0.03 ^{Bd}	0.30	22
A2	0.42 ±0.23 ^{Acde}	0.60 ±0.15 ^{Ab}	0.68 ±0.14 ^{Abcd}	1.70	15
A3	0.07 ±0.03 ^{Bde}	0.27 ±0.19 ^{Ab}	0.34 ±0.05 ^{Acd}	0.68	21
A4	1.69 ±0.75 $^{Aa–e}$	0.32 ±0.11 ^{Bb}	1.59 ±0.19 ^{Aa}	3.60	7
A5	1.94 ±0.51 ^{Aabc}	0.54 ±0.28 ^{Bb}	0.24 ±0.13 ^{Bcd}	2.72	9
A6	0.64 ±0.16 ^{Acde}	0.51 ±0.14 ^{Ab}	0.00 ±0.00 ^{Bd}	1.15	17
A7	0.77 ±0.11 ^{Bcde}	1.37 ±1.08 ^{Ab}	0.60 ±0.19 ^{Bbcd}	2.74	9
A8	0.75 ±0.20 ^{Bcde}	1.37 ±0.36 ^{Ab}	1.83 ±0.37 ^{Aa}	3.95	5
A9	2.74 ±0.79 ^{Aab}	2.86 ±0.99 ^{Aa}	1.21 ±0.64 ^{Bab}	6.81	2
A10	1.21 ±0.38 $^{Ab–e}$	0.10 ±0.10 ^{Bb}	0.02 ±0.02 ^{Bd}	1.33	16
A11	0.47 ±0.25 ^{Acde}	0.37 ±0.13 ^{Ab}	0.00 ±0.00 ^{Bd}	0.84	19
A12	1.03 ±0.50 ^{Acde}	0.44 ±0.26 ^{Bb}	0.35 ±0.35 ^{Bcd}	1.82	13
A13	1.70 ±0.45 $^{Aa–e}$	0.65 ±0.19 ^{Bb}	0.14 ±0.12 ^{Ccd}	2.49	12
A14	0.48 ±0.16 ^{Acde}	0.32 ±0.23 ^{Ab}	0.04 ±0.03 ^{Bd}	0.84	19
A15	0.91 ±0.46 ^{Bcde}	0.00 ±0.00 ^{Cb}	1.60 ±0.72 ^{Aa}	2.51	11
A16	1.67 ±0.31 $^{Aa–e}$	1.41 ±0.30 ^{Ab}	0.69 ±0.18 ^{Bbcd}	3.77	6
A17	0.00 ±0.00 ^{Ae}	0.00 ±0.00 ^{Ab}	0.00 ±0.00 ^{Ad}	0.00	23
A18	1.75 ±0.51 $^{Aa–d}$	1.04 ±0.36 ^{Bb}	0.15 ±0.08 ^{Ccd}	2.94	8
A19	0.63 ±0.47 ^{Acde}	0.22 ±0.11 ^{Ab}	0.23 ±0.10 ^{Acd}	1.08	18
A20	0.45 ±0.20 ^{Bcde}	0.30 ±0.19 ^{Bb}	1.02 ±0.14 ^{Aabc}	1.77	14
A21	1.76 ±0.35 $^{Aa–d}$	1.12 ±0.09 ^{Ab}	1.26 ±0.19 ^{Aab}	4.14	4

* D – diploid; T – triploid; A1 to A21 – aneuploid. The codes are consistent with those in Tab. 2. ** Data are provided as mean ±*SE*. Values in each column followed by different letters are significantly different at $p < 0.05$. The uppercases represent the significant differences among the different explants of the same line. The lowercases represent the significant differences among the same explants of different lines.

triploid (2.85) parents. Six aneuploids, including A5 (1.94), A21 (1.76), A18 (1.75), A13 (1.7), A4 (1.69), and A16 (1.67), presented similar and slightly lower regeneration rates than A9. Other aneuploids, e.g., A10 and A12, had lower regeneration rates than the aneuploids mentioned above. For petiole explants, an aneuploid-A9 (2.86) showed a higher regeneration rate than the triploid parent (1.25) and comparable to the diploid parent (2.87). Five aneuploids, including A16 (1.41), A7 (1.37), A8 (1.37), A21 (1.12), and A18 (1.04), had regeneration rates close to that of the triploid parent (1.25). Other aneuploids, e.g., A13 and A2, had poorer regeneration rates than the above aneuploids. For root explants, an aneuploid, A8 (1.83), showed a higher regeneration rate than the diploid parent (1.69). Five aneuploids, including A15 (1.6), A4 (1.59), A21 (1.26), A9 (1.21), and A20 (1.02), had higher regeneration rates than that of the triploid parent (0.7); three aneuploids, including A16 (0.69), A2 (0.68), and A7 (0.6), showed comparable

regeneration rates to the triploid parent (0.7). The remaining 12 aneuploids had poorer regeneration rates than the above aneuploids. Although most aneuploids had poorer regeneration rates than the diploid and triploid parents, adventitious bud regeneration rates could be improved by modifying the medium composition. For example, aneuploid A17 did not regenerate buds on MS medium supplemented with 0.4 mg/L BA like all other aneuploids, but could regenerate some buds after supplementation with 0.7 mg/L BA (unpublished data).

Comparison of in vitro plantlet morphology among aneuploids and their diploid and triploid parents

Large morphological variations were observed among aneuploids and their parents (Fig. 3). Aneuploids A1, A7, A13, A18, A19, A20, and A21 had markedly longer petioles, while aneuploids A10 and A15 had very short petioles. A11 had clearly thinner roots while A3, A10, and A21 had thicker and shorter roots. The plantlets of A15 were very tiny and in light green, while the plantlets of A10 were in darker green pigmentation than others. Compared with diploid and triploid parents, almost half of the aneuploids (A1, A2, A3, A4, A7, A8, A9, A16, A18, A20, and A21) looked morphologically normal and presented higher survival rates than others when transplanted to ex vitro conditions.

Comparison of main bioactive compounds contents among aneuploids and their diploid and triploid parents

Contents of cichoric acid (Tab. 4) and chlorogenic acid (Tab. 5) were compared among aneuploids and their parents. Other compounds, including echinacoside, caftaric acid, and 1,3-dicaffeoylquinic acid, were detected in trace amounts (unpublished data).

Cichoric acid contents in underground parts were higher than those of aboveground parts for almost all aneuploids except that A3 which had lower cichoric acid content in underground part (9.81 mg/g) than in aboveground part (10.65 mg/g). Most aneuploids (e.g., A2, A3, A5, A20, A7, A13, A21, A9, and A10) had higher cichoric acid contents than the parents; A2 showed the highest amount of 21.98 mg/g, which was more than twice the values obtained in the diploid and triploid parents. The contents of chlorogenic acid were lower than cichoric acid levels. Similarly, chlorogenic acid was accumulated mainly in underground parts, and most aneuploids (e.g., A21, A5, A10, A12, A7, A2, and A18) had higher chlorogenic acid contents than the parents; A21 exhibited the highest chlorogenic acid content of 1.84 mg/g, which was approximately five times higher than the contents in the diploid and triploid parents.

Discussion

The chromosome numbers of aneuploids obtained in reciprocal crosses between diploid and triploid were widely distributed from 23 to 31 (Tab. 1, Tab. 2), indicating that chromosome numbers of the gametes produced in *E. purpurea* triploid were evenly distributed from 12 to 20. This contrasted with data reported in foxtail millet [36] and cucumber [37] that produced mainly trisomy. It is worth mentioning that both reciprocal crosses yielded polyploids such as tetraploid. This might result from the production of unreduced gametes caused by interploidy hybridization [28,30]. The presence of other euploid individuals, e.g., diploid and triploid, indicated that triploid could also produce euploid gametes of $n = 11$ and 22. Many more seeds were obtained in crosses with diploid as the female parent (Tab. 1). The difference might be due to very intensively distributed florets in one inflorescence (Fig. 1), which causes stigmas of the diploid female parent to be easily contaminated by other diploid pollens during the pollination process. Moreover, interploidal hybridization has strong reproductive isolation [38,39], so diploid pollens are more competitive than that of triploid. Using triploid as the female parent resulted in increased production of aneuploid individuals (19 of 23; 82.61%) compared with crossing involving a diploid female parent (Tab. 1).

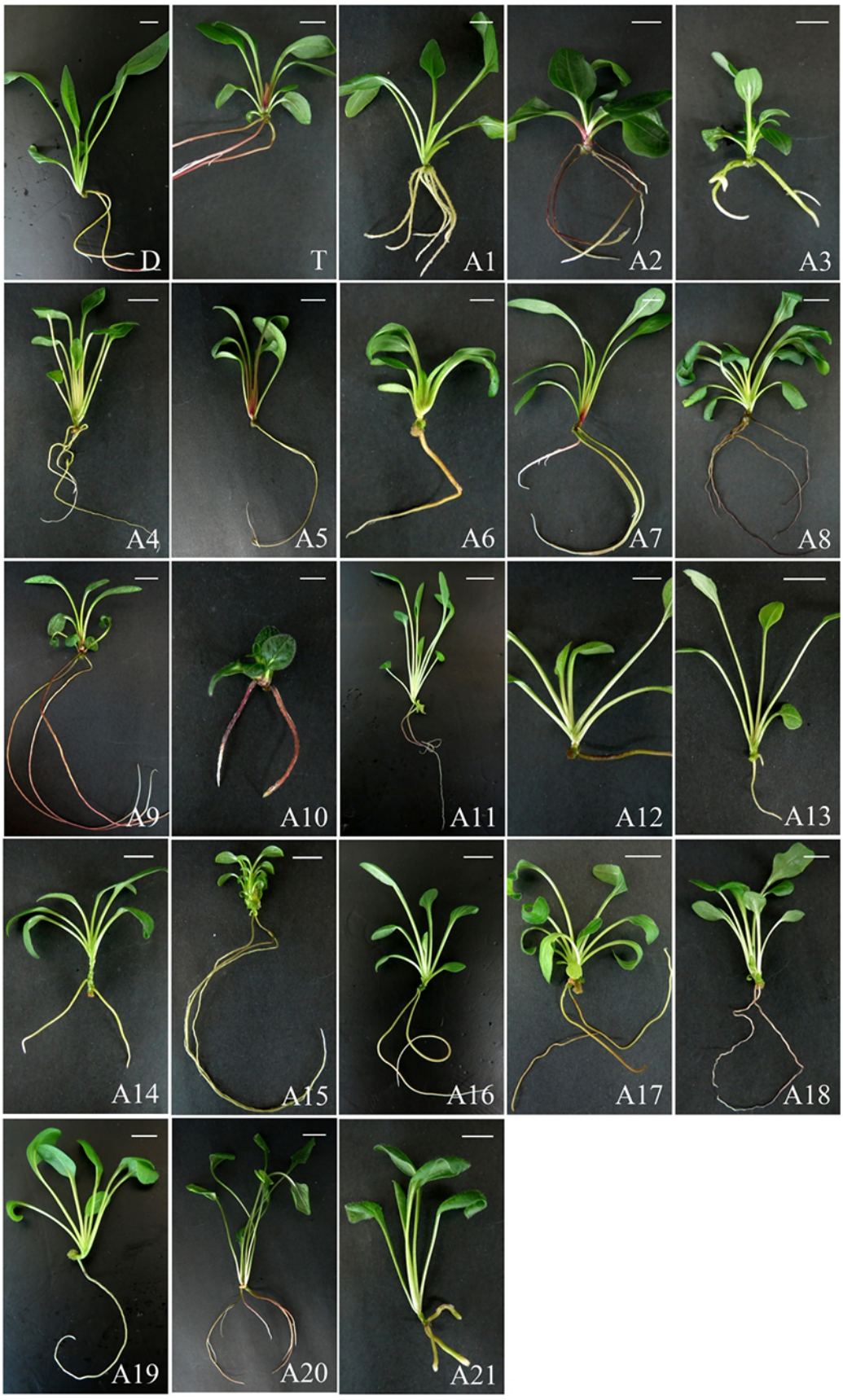

Fig. 3 Comparison of morphology of in vitro plantlets among aneuploids and their diploid and triploid parents in *Echinacea purpurea* L. A representative in vitro plantlet is selected in each aneuploid. Codes in the photographs are consistent with those in Tab. 2. Scale bar: 1 cm.

Tab. 4 Comparison of cichoric acid contents among aneuploid progenies and their diploid and triploid parents in *Echinacea purpurea* L.

	Aboveground part		Underground part		Sum	
Code*	mg/g	ranking	mg/g	ranking	mg/g	ranking
D	2.89 ±0.63 $^{d–i}$**	11	5.94 ±1.97 ^{def}	16	8.83	15
T	2.40 ±0.20 $^{f–i}$	14	6.47 ±1.76 ^{def}	15	8.87	14
A1	2.26 ±0.06 ^{ghi}	15	4.52 ±0.42 ^{ef}	18	6.78	18
A2	6.73 ±0.34 ^{b}	2	15.25 ±0.74 ^{a}	2	21.98	1
A3	10.65 ±1.52 ^{a}	1	9.81 ±1.57 ^{bcd}	7	20.46	2
A4	1.33 ±0.08 ^{i}	20	8.28 ±1.03 ^{cde}	11	9.61	13
A5	3.80 ±1.11 $^{c–h}$	10	15.59 ±0.16 ^{a}	1	19.39	3
A6	4.36 ±0.97 $^{c–f}$	8	7.22 ±0.34 ^{def}	13	11.58	11
A7	5.29 ±0.10 ^{bc}	4	12.20 ±1.01 ^{abc}	5	17.49	5
A8	1.77 ±0.33 ^{hi}	18	6.80 ±1.87 ^{def}	14	8.57	16
A9	4.88 ±0.60 ^{bcd}	5	9.45 ±0.06 ^{cd}	8	14.33	8
A10	4.82 ±0.89 ^{bcd}	6	7.24 ±1.47 ^{def}	12	12.06	9
A11	1.69 ±0.31 ^{i}	19	3.20 ±0.10 ^{f}	19	4.89	19
A12	2.74 ±0.16 $^{e–i}$	12	8.67 ±1.20 ^{cde}	10	11.41	12
A13	4.53 ±0.72 ^{cde}	7	12.79 ±0.50 ^{abc}	4	17.32	6
A14	- ***	-	-	-	-	-
A15	-	-	-	-	-	-
A16	1.91 ±0.15 ^{hi}	16	2.87 ±0.23 ^{f}	20	4.78	20
A17	-	-	-	-	-	-
A18	1.84 ±0.06 ^{hi}	17	10.05 ±1.02 ^{bcd}	6	11. 99	10
A19	2.66 ±0.10 $^{e–i}$	13	5.51 ±0.83 ^{def}	17	8.17	17
A20	3.99 ±0.29 $^{c–g}$	9	14.05 ±0.59 ^{ab}	3	18.04	4
A21	5.77 ±0.44 ^{bc}	3	9.05 ±1.18 ^{cde}	9	14.82	7

* D – diploid; T – triploid; A1 to A21 – aneuploid. The codes are consistent with those in Tab. 2. ** Data are provided as mean ±*SE*. Values in each column followed by different letters are significantly different at $p < 0.05$. *** Data not available due to insufficient sample plant materials.

Besides diploid stigmas were contaminated by other diploid pollens in the case of a female diploid parent, this might also be affected by the aberrant seed development in interploidy crosses. The discriminating embryo and endosperm development between reciprocal interploidy crosses have been well studied [38–40]. Endosperm development is more affected by parental gene dosage changes than embryo and presents contrasting phenotypes between reciprocal interploidal hybridizations [39]. Maternal excess cross resulted in early cellularization and poor proliferation of endosperm, while paternal excess cross leaded to extend cellularization and over proliferation of endosperm [38,39]. Further studies are required to verify these differences in *E. purpurea*.

All aneuploids investigated in the present study had particular karyotypic characteristics. Although they had the same chromosome types (m, sm, and st) as the parents, their proportions were largely different (Tab. 2). Almost all chromosomes in 21 aneuploids looked morphologically normal, except that two chromosomes in A3 and A18 were

Tab. 5 Comparison of chlorogenic acid contents among aneuploid progenies and their diploid and triploid parents in *Echinacea purpurea* L.

	Aboveground part		Underground part		Sum	
Code*	mg/g	ranking	mg/g	ranking	mg/g	ranking
D	0.05 ±0.03 ^{def}**	10	0.20 ±0.09 ^{ghi}	17	0.25	18
T	0.00 ±0.00 ^{f}	13	0.34 ±0.11 $^{e–i}$	14	0.34	15
A1	0.00 ±0.00 ^{f}	13	0.38 ±0.07 $^{e–i}$	12	0.38	13
A2	0.12 ±0.02 $^{b–f}$	7	0.89 ±0.13 ^{bcd}	6	1.01	6
A3	0.28 ±0.02 ^{b}	2	0.51 ±0.00 $^{d–h}$	10	0.79	9
A4	0.00 ±0.00 ^{f}	13	0.42 ±0.34 $^{d–i}$	11	0.42	12
A5	0.00 ±0.00 ^{f}	13	1.49 ±0.08 ^{ab}	2	1.49	2
A6	0.08 ±0.04 $^{c–f}$	8	0.63 ±0.20 $^{c–g}$	9	0.71	10
A7	0.13 ±0.01 $^{b–f}$	6	1.03 ±0.20 ^{bc}	4	1.16	4
A8	0.00 ±0.00 ^{f}	13	0.30 ±0.14 $^{f–i}$	15	0.30	17
A9	0.05 ±0.05 ^{def}	10	0.20 ±0.05 ^{ghi}	17	0.25	18
A10	0.08 ±0.04 $^{c–f}$	8	1.18 ±0.15 ^{b}	3	1.26	3
A11	0.00 ±0.00 ^{f}	13	0.22 ±0.01 ^{ghi}	16	0.22	20
A12	0.26 ±0.05 ^{bc}	3	0.90 ±0.15 ^{bcd}	5	1.16	4
A13	0.05 ±0.03 ^{def}	10	0.79 ±0.04 $^{b–f}$	8	0.84	8
A14	- ***	-	-	-	-	-
A15	-	-	-	-	-	-
A16	0.24 ±0.08 ^{bcd}	4	0.10 ±0.02 ^{hi}	19	0.34	15
A17	-	-	-	-	-	-
A18	0.00 ±0.00 ^{f}	13	0.85 ±0.13 $^{b–e}$	7	0.85	7
A19	0.53 ±0.25 ^{a}	1	0.00 ±0.00 ^{i}	20	0.53	11
A20	0.00 ±0.00 ^{f}	13	0.37 ±0.06 $^{e–i}$	13	0.37	13
A21	0.22 ±0.02 $^{b–e}$	5	1.62 ±0.14 ^{a}	1	1.84	1

* D – diploid; T – triploid; A1 to A21 – aneuploid. The codes are consistent with those in Tab. 2. ** Data are provided as mean ±*SE*. Values in each column followed by different letters are significantly different at $p < 0.05$. *** Data not available due to insufficient sample plant materials.

markedly shorter than others (Fig. 2). However, many other chromosome changes occurred (e.g., chromosome deletions, duplications, translocations, and inversions) could not been identified with the method used in present study. Aneuploid might also involve genome changes and gene expression perturbations [3,4]. All these changes resulted in various performances of aneuploids, and the similar findings had been reported in previous study [5]. All the variations in chromosomes and genomes were needed to verify with more precise analytical approaches in *E. purpurea* aneuploids.

As aneuploids could not be propagated through sexual reproduction, in vitro cloning was an important alternative method. Aneuploid progenies had diverse adventitious bud regeneration rates compared with the parents (Tab. 3). Although adventitious bud regeneration rates of aneuploid progenies were relatively lower compared with parental values, modifying medium compositions could enhance regeneration efficiency [41–44]. Morphology of in vitro plantlet directly determined the survival rate while transplanting

to ex vitro conditions. Almost half of the aneuploid progenies had developed roots and spread phenotypes (Fig. 3), this would aid transplanting to ex vitro conditions.

Among the five kinds of caffeic acid derivatives detected, the content of cichoric acid was the highest, and followed by chlorogenic acid. Echinacoside, caftaric acid, and 1,3-dicaffeoylquinic acid were detected in trace amounts. Both cichoric acid and chlorogenic acid were mainly accumulated in underground parts. They were similar in 21 aneuploids and two parents (Tab. 4, Tab. 5). Thirteen aneuploids had higher whole-plant cichoric acid contents than two parents, and the contents of A2 and A3 were more than twice the values of diploid and triploid parents. Fourteen aneuploids had higher whole-plant chlorogenic acid contents than parents, with A21 exhibiting the highest content which was approximately five times higher than the contents of diploid and triploid parents. Enhanced production of cichoric acid and chlorogenic acid were also reported in *E. purpurea* tetraploid plants compared with diploid individuals [24,25,45], but not to the extent found in aneuploids, e.g., Abdoli detected that leaves of tetraploid plants had 45% and 71% more cichoric acid and chlorogenic acid than diploid plants [45].

Based on Fig. 3 and Tab. 3–Tab. 5 data, *E. purpurea* seems to be a plant species with great tolerance of chromosome constitution imbalance. This notion is also supported by the fact that aneuploid plants are frequently found in Asteraceae [7], the family to which *E. purpurea* belongs. These findings provide a great opportunity for using *E. purpurea* aneuploids as unique cultivated resources for special genetic studies and breeding purposes. Because cichoric acid is the main medicinal compound in *E. purpurea* [46,47], its content should be considered preferentially when screening superior lines from aneuploids. Aneuploids that showed abnormal morphologies (e.g., A5 and A6) must be abandoned to ensure survival rates while transplanting to ex vitro conditions. Based on the above demonstrated characteristics, the aneuploids A1, A2, A3, A4, A7, A8, A9, A16, A18, A20, and A21 appear to be competent for further selection. In future work, further assessments of gene expression mechanisms in *E. purpurea* aneuploids should be carried out to explore more meaningful discovery.

Supplementary material

The following supplementary material for this article is available at http://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.3556/0:

Fig. S1 The original chromosomal images of aneuploid progeny seedlings and their diploid and triploid parents in *Echinacea purpurea* L.

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