Chapter 16

Plant Responses at Different Ploidy Levels

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1. Introduction

The term "ploidy" expresses the number of sets of chromosomes in a biological cell and marked by an "X". A diploid genotype carries two paired (homologous) sets of chromosomes in the nucleus of each cell, one from each parent (Figure 1a). "Polyploidy" is the multiplication of entire sets of chromosomes. In other words, polyploid genotype has more than two homologous sets of chromosomes in its cell. For example, tetraploid plants have four sets of chromosomes in their cells (Figure 1b). Polyploidy is common among flowering plants (angiosperms) and is a major force in plant speciation [1]. Almost 47%-70% of angiosperms are polyploid [1-3].

There are differences between diploid and polyploid plants from morphological, physiological, cellular and biochemical aspects. Polyploid plants have bigger cells and stomatas than diploid ones that result in thicker and big leaves, larger flowers and fruits. In general, autotetraploids have greater vegetative volume and larger seed weight but lower reproductive fertility than diploids, and flowering and fruit formation were often later in tetraploids than in diploids as reported by Stebbins [4]. Shoots of polyploid genotypes are thicker with short internodes and wider crotch angles. As the chromosome number increased, DNA content per cell, enzyme activity per cell and cell volume all increased [5, 6]. In addition, polyploids are used as sources of variability and new genotypes for plant improvement [7, 8].

Polyploid genotypes have shown resistance to biotic (pests and pathogens) and abiotic (drought and cold etc.) stress factors in some cases and this resistance enables them to have greater adaptability to wider ecological regions. This could be attributed to higher chromosome number and gene expression causing to increase in the concentration of particular secondary metabolites and chemicals that are responsible for defense mechanism. This increase in the concentration of particular secondary metabolites and chemicals enable polyploid genotypes to resist against biotic and abiotic stress factors, consequently to grow in the wide range of environments.

However, the effects of increased ploidy level cannot be anticipated all the time. In contrast to common knowledge that polyploid individuals are superior than the diploid ones from many aspects, in some cases polyploid plants can have slower growth rates [9] which could be attributed to difficulties in the cell cycle and slow cell division [10] causing to fewer cell number and smaller organs. For example, it was reported that the overall chlorophyll content in polyploid plants are higher than diploid ones with lower chromosome numbers [5, 6, 11], while chlorophyll a, chlorophyll b and total chlorophyll contents of tetraploid sugar beet genotypes ('AD 440' and 'CBM 315') in our study were found to be lower than diploid ones ('Agnessa' and 'Felicita') [12].

Although there are studies reporting that seeds of tetraploid plants germinated faster with a higher percentage than those of diploids [13], in the study we conducted under greenhouse conditions, it was observed that germination and seedling growth of diploid sugar beet genotypes were much better than tetraploids. Our findings were parallel to the ones reporting that polyploid seeds might show lower germination and emergence percentage than diploids and this could be attributed to thicker seedcoat and weak seedling emergence [14] and weak embryo development [15].

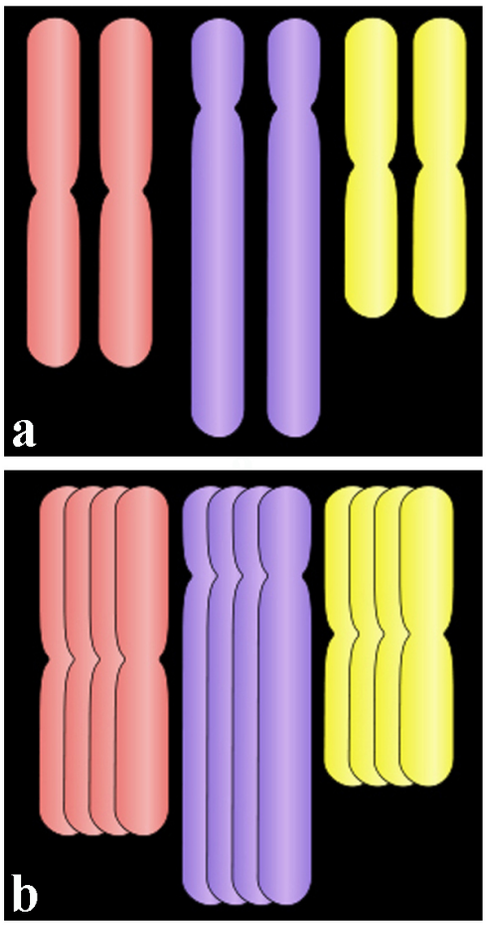

Figure 1. Diagram for cells at different ploidy levels. (a) Diploid (indicated by 2n = 2X) cell having two homologous copies of each chromosome, (b) Tetraploid (indicated by 2n = 4X) cell having four homologous copies of each chromosome

2. Growth pattern of genotypes at different ploidy levels

In a study conducted in sugar beet which is an important sucrose-producing crop worldwide in temperate regions and supplies about 20% of the world sugar consumption, two diploid ('Agnessa' and 'Felicita') and two tetraploid ('AD 440' and 'CBM 315') sugar beet genotypes were compared with respect to vegetative and generative characteristics such as seed germination, seedling growth, total chlorophyll and protein contents, root and sugar yields, and sugar content [12]. The size of epidermal cells in a field of view area on upper leaf surface of sterile seedlings were counted using clear fingernail polish, clear tape, a glass slide, and a microscope at 60X magnification. From these counts, the highest results regarding cell size were recorded in tetraploid genotypes (Table 1, Figure 2). Decreased cell number in polyploid genotypes was compensated for by increased cell size as reported by Doonan [16] and, Inze and De Veylder [17].

Genotypes	Cell Number	Cell Length (µm)	Cell Width (µm)	Approx. Cell Area (µm²)
'Agnessa' (2X)	167.65 a	86.52 b	31.24 b	2702.88 b
'Felicita' (2X)	152.80 a	82.14 b	33.16 b	2723.76 b
'AD 440' (4X)	78.60 b	131.62 a	48.42 a	6373.04 a
'CBM 315' (4X)	72.40 b	128.24 a	52.16 a	6688.99 a

Values followed by the different letters in a column are significantly different at the 0.01 level

Table 1. Cell sizes in the upper leaf surface of 6-week-old sugar beet plants at different ploidy levels

Diploid genotypes gave higher results than tetraploids in seed germination percentage, root length and seedling height at 4th day and root length and seedling height at 14th day (Table 2). Polyploid seeds had lower germination and emergence percentages than diploid ones due to their thicker seedcoat and seedling emergence strength [14, 15].

In the first 6 weeks, diploid genotypes gave rise to the highest results with respect to plant height, root lenght, leaf length and width, approx. leaf area, plant fresh and dry weights, total chlorophyll content and protein percentage (Figure 3, Table 3). However, they were passed by tetraploid genotypes in the further stages of the development in the characters of plant height, root lenght, leaf length and width, approx. leaf area, plant fresh and dry weights (Figure 4, Table 4). These figures showed that tetraploid genotypes passed diploids vegetatively in the further developmental stages.

Plants developed from diploid seeds were more vital and well-grown. Plant height and root length scores in diploid genotypes were good indicators for vitality and growth. Leaf area which plays an important role on the photosynthetic acticity, was found higher in diploid genotypes in the first 6 weeks. High ploidy level does not result in increased shoot growth every time [18].

It was reported that the fresh weight increase was mainly due to cell enlargement by water absorption [19] and increase in dry weight was closely related to cell division and new material synthesis [20]. Dry weight increase in diploids was due to an increase in photosynthetic activity and carbohydrate metabolism resulting from increased water uptake by longer roots. Reduced fresh weight in tetraploids could be attributed to decreased water absorption as reported by Prado et al. [21]. Sullivan and Phafter [22] reported that seedling growth was affected by genotypic differences more than ploidy in diploid and autotetraploid *Secale cereale*. Lower results in morphological characters in the first developmental stages of tetraploid genotypes could be attributed to slow cell division as reported by Comai [10].

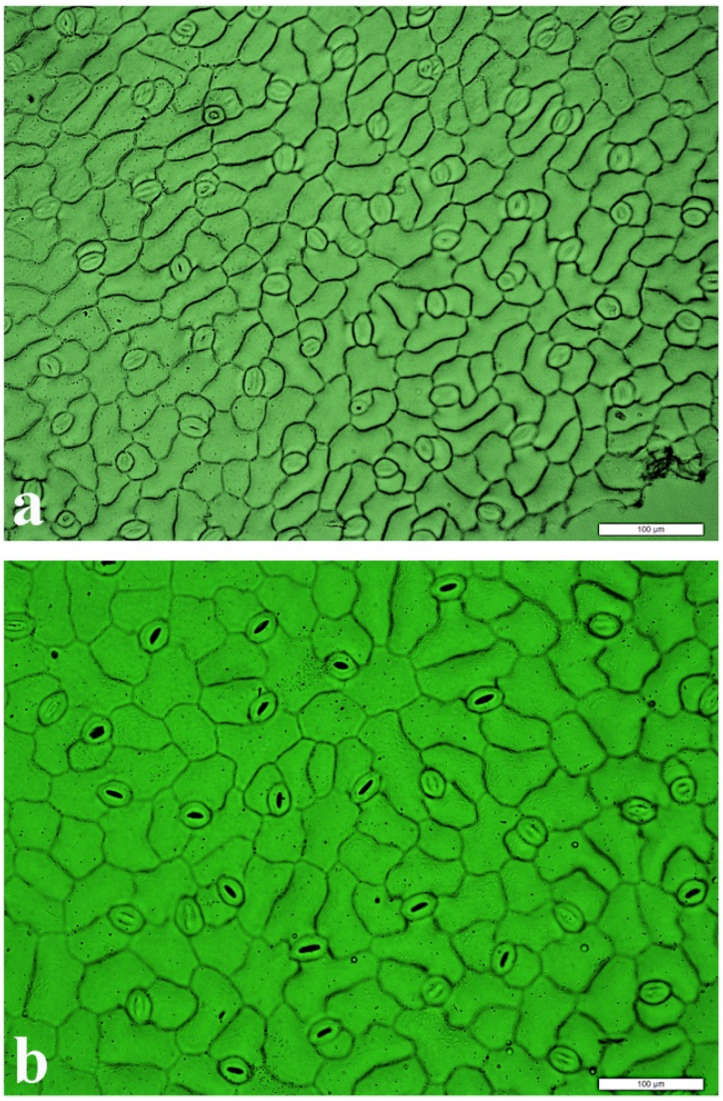

Figure 2. Cells and stomatas from the upper leaf surface of 6-week-old sugar beet seedlings. (a) Diploid genotype 'Felicita' and (b) Tetraploid genotype 'AD 440'

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Genotypes	DAY 4			DAY 14	
	Germination (%)	Root Length (cm)	Seedling Height (cm)	Root Length (cm)	Seedling Height (cm)
'Agnessa' (2X)	75.10 a	7.40 a	6.34 a	12.67 a	9.05 a
'Felicita' (2X)	89.10 a	8.45 a	7.00 a	13.96 a	9.52 a
'AD 440' (4X)	60.10 b	5.05 b	5.74 a	7.50 b	6.21 b
'CBM 315' (4X)	60.10 b	5.86 b	5.49 a	7.52 b	7.38 b

Values followed by the different letters in a column are significantly different at the 0.01 level

Table 2. Germination and seedling growth in diploid and tetraploid sugar beet genotypes

Chlorophyll content which is accepted as an indicator of photosynthetic capacity of tissues [23-25], was again found higher in diploid plants. It was thought that this could be due to the fact that photosynthetic capacity of the tissue in diploids was higher because of higher chlorophyll content, water and nutrient uptake from the soil with their roots. Higher photosynthetic capacity resulted in higher protein content in diploids. The number of phosynthetic cells per unit leaf area decreases with increasing ploidy level [26]. Although chloroplasts [27, 28] and chlorophyll content [5] are higher in polyploid genotypes, increase tendency of chlorophyll content by increasing ploidy level is not always apparent. For instance, chlorophyll content remained constant in different ploidy levels of *Atriplex confertifolia* [26].

Polyploid plants may show high-ploidy syndrome that could be explained by costly cell cycle and slow cell division at higher ploidy levels. That means in some cases, diploid genotypes can show superior characteristics than tetraploid ones.

Figure 3. Development of seedlings from seeds of (a) diploid ('Felicita') and (b) tetraploid ('AD 440') genotypes 6 weeks after study initiation (Bar = 3 cm)

Genotypes	Vegetative Characters							Generative Characters	
	Plant Height (cm)	Root Length (cm)	Leaf Length (cm)	Leaf Width (cm)	Approx. Leaf Area (cm^2)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Total Chlorophyll Content (µg/g fresh tissue)	Protein (%)
'Agnessa' (2X)	18.53 a	21.40 a	5.13 a	3.04 a	16.51 a	11.81 a	2.50 a	894.07 b	18.45 a
'Felicita' (2X)	19.47 a	19.34 a	5.75 a	3.14 a	18.06 a	9.10 a	2.78 a	1035.47 a	21.07 a
Mean	19.00	20.37	5.44	3.09	17.28	10.45	2.64	964.77	19.76
'AD 440' (4X)	15.01 b	13.46 b	5.00 a	2.34 b	11.70 b	6.19 b	1.18 b	815.99 b	6.33 b
'CBM 315' (4X)	15.14 b	14.95 b	4.96 a	2.49 b	12.35 b	4.69 b	0.94 b	679.82 c	2.90 b
Mean	15.07	14.20	4.98	2.41	12.02	5.44	1.06	747.90	4.61

Values followed by the different letters in a column are significantly different at the 0.01 level

Table 3. Development of seedlings from seeds of diploid and tetraploid genotypes 6 weeks after study initiation

Figure 4. Development of seedlings from seeds of (a) diploid ('Felicita') and (b) tetraploid ('AD 440') genotypes 10 weeks after study initiation (Bar = 5 cm)

In general, tetraploids have higher vegetative growth but lower reproductive fertility than diploids. Thus, in our study, tetraploid genotypes passed diploid ones 10 weeks after study initiation regarding vegetative characters such as plant height, root length, leaf length and width, approximate leaf area, plant fresh and dry weights [12]. Data related to generative characters such as total chlorophyll content and protein percentage were the highest in diploid

genotyes (Table 4). Root and sugar yields, and sugar content obtained from field trials 6 months after study initiation were again found the highest in diploids (Table 5). Polyploids flower and fruit later than diploids as reported by Stebbins [4].

Genotypes	Vegetative Characters							Generative Characters	
	Plant Height (cm)	Root Length (cm)	Leaf Length (cm)	Leaf Width (cm)	Approx. Leaf Area (cm^2)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Total Chlorophyll Content (µg/g fresh tissue)	Protein (%)
'Agnessa' (2X)	20.5 b	29.0 b	13.4 b	7.3 b	97.8 b	286.6 b	42.9 b	1571.5 a	20.46 a
'Felicita' (2X)	27.3 b	33.8 b	14.9 b	6.9 b	102.8 b	306.1 b	51.6 b	1533.8 a	21.77 a
Mean	23.90	31.40	14.15	7.10	100.30	296.35	47.25	1552.65	21.11
'AD 440' (4X)	37.0 a	42.9 a	18.7 a	9.7 a	181.4 a	357.1 a	77.3 a	1210.6 b	16.10 b
'CBM 315' (4X)	33.0 a	44.8 a	18.1 a	8.8 a	159.3 a	381.1 a	77.6 a	1276.5 b	17.39 b
Mean	35.00	43.85	18.40	9.25	170.35	369.10	77.45	1243.55	16.74

Values followed by the different letters in a column are significantly different at the 0.01 level

Table 4. Development of seedlings from seeds of diploid and tetraploid genotypes 10 weeks after study initiation

Genotypes	Root Yield (tones/ha)	Sugar Content (%)	Sugar Yield (tones/ha)
'Agnessa' (2X)	74.46 a	14.88 a	11.08 a
'Felicita' (2X)	68.48 a	16.21 a	11.10 a
'AD 440' (4X)	54.40 b	12.85 b	6.99 b
'CBM 315' (4X)	58.66 b	12.07 b	7.08 b

Values followed by the different letters in a column are significantly different at the 0.01 level

Table 5. Sugar content, root and sugar yields in diploid and tetraploid genotypes

3. Regeneration capacity of genotypes at different ploidy levels under *In vitro* conditions

Genetic variation is a prerequisite for successful plant breeding. *In vitro* culture techniques seem to offer certain advantages in this respect through somatic hybridization, induction of mutants and selection of disease free and disease resistant plants [29].

In a study conducted in sugar beet, it was aimed to examine the effect of the ploidy level on *in vitro* explant growth, adventitious shoot regeneration, rooting and plantlet establishment from petiole segments of two inbred lines ('ELK 345' - diploid and 'CBM 315' - tetraploid) [30]. Petioles were used as explant and 1 mg l^{-1} BAP and 0.2 mg l^{-1} NAA as the combination of growth regulators for shoot regeneration in accordance with studies reporting that the most responsive explant for *in vitro* culture of sugar beet was petiole [31-37] and the combination of the plant growth regulators was 1 mg l^{-1} BAP and 0.2 mg l^{-1} NAA [37]. The results clearly showed that there were sharp and statistically significant differences in all parameters examined between lines at different ploidy levels. The study was set in three parallels to confirm the accuracy of the study.

The tetraploid line 'CBM 315' had a higher fresh weight than the diploid line 'ELK 345' in all three experiments (Table 6). In all experiments, the differences in fresh weight between the diploid and the tetraploid lines were statistically significant ($p < 0.05$). Dry weight scores were again found to be higher in the tetraploid line, and the differences between these lines were statistically significant at 0.01 level in all experiments (Table 6). The highest mean of fresh and dry weights of petiole explants was recorded from the tetraploid line as 0.254 g and 0.023 g. In the diploid line, the mean fresh and dry weights of petioles were noted as 0.172 g and 0.012 g (Table 6). The difference between fresh and dry weights signifies the tissue water content. From these results, the tissue water content was calculated as 0.231 g (0.254-0.023) in the tetraploid line 'CBM 315', and 0.160 g (0.172-0.012) in the diploid line 'ELK 345'.

The cells with high ploidy levels have bigger vacuoles [38] that play an important role in regulating the osmotic pressure of the cell [39]. Higher osmotic pressure of the cell in polyploid plants, as reported by Tal and Gardi [40], could cause higher tissue metabolic activity by increasing water and hormone uptake from the medium. Cell enlargement by water absorption, cell vacuolation, and turgor-deriven wall expansion is the main reason of fresh weight increase, as reported by Dale [19]. The increase in dry weight was closely related to cell division and new material synthesis [20]. Thus, increase in the fresh and dry weights of petiole explants of the tetraploid line in our study at the end of culture were chiefly due to an increase in the absorption of water and other components from the basal medium via the high cell osmotic pressure. On the contrary, lower osmotic pressure of the diploid line caused a decline in fresh and dry weights of petioles by decreasing the absorption of water and other components from the medium. Results about tissue water content clearly showed that the tetraploid line had higher osmotic pressure, which caused higher absorption of water and other components from the medium. Higher results of all parameters in the study could be attributed to higher cell osmotic pressure of the tetraploid line 'CBM 315'. Yildiz and Ozgen [41] have reported that increasing tissue water content, which caused higher tissue metabolic activity, resulted in higher results of all parameters examined.

The increase in ploidy level leads to a larger cell that has a higher growth rate [38]. Tetraploid genotypes had a higher water content [40] and more organic solutes than diploid genotypes [42]. Warner and Edwards [26] have reported that the chromosome number determines the size of leaves, the size of cells, the number of chloroplasts per cell, and amounts of photosynthetic enzymes and pigments in cell. As the chromosome number increased, DNA content per

		Fresh Weight (g)		Dry Weight (g)		Water Content (%)		Dry Matter Content (%)	
		'ELK 345' 2X	'CBM 315' 4X	'ELK 345' 2X	'CBM 315' 4X	'ELK 345' 2X	'CBM 315' 4X	'ELK 345' 2X	'CBM 315' 4X
1st experiment		0.168±0.009	0.226±0.011	0.012±0.001	0.023±0.002	92.83±0.410	89.76±0.410	7.17±1.147	10.24±1.147
	t value	4.226*		4.621**		2.561*		2.561*	
2nd experiment		0.161±0.091	0.251±0.028	0.012±0.001	0.022±0.002	92.34±0.361	91.34±0.275	7.66±0.361	8.66±0.275
	t value	3.120*		4.542**		2.165ns		2.165ns	
3rd experiment		0.187±0.017	0.284±0.019	0.013±0.0003	0.023±0.001	92.76±0.654	91.77±0.258	7.24±0.654	8.23±0.258
	t value	3.794*		6.708**		1.401ns		1.401ns	
Mean[1]		0.172	0.254	0.012	0.023	92.64	90.96	7.36	9.04

Significantly different from zero at * $p < 0.05$ and ** $p < 0.01$

[1] Mean of three experiments

Table 6. Fresh and dry weights, water and dry matter contents of petiole explants of 'ELK 345' (diploid) and 'CBM 315' (tetraploid) lines 5 weeks after culture initiation on MS medium containing 1 mg l^{-1} BAP and 0.2 mg l^{-1} NAA

cell, enzyme activity per cell, cell volume, and photosynthesis per cell all increased. It was also reported that the photosynthetic capacity of larger cells in polyploid plants are higher than smaller cells with lower chromosome numbers [5, 6, 43].

In all experiments, the highest results were obtained from petiole explants of the tetraploid line in the parameters of shoot regeneration percentage, shoot number per petiole, shoot length, total shoot number per Petri dish, number of shoots rooted, and the percentage of shoots rooted. During culture, petiole explants of the tetraploid line were observed to grow faster than the ones of the diploid line. By the end of the culture, petiole explants of the tetraploid line were bigger and well developed, and the number of shoots regenerated was also higher (Figure 5a-b) than the diploid line (Figure 5c). The differences between petiole explants of the diploid and tetraploid lines for all parameters examined were statistically significant at $p < 0.01$, with the exception of shoot regeneration percentage in all experiments which was different at $p < 0.05$ (Table 7, Table 8) [30].

Shoot primordias on petiole explants appeared in the first week of the culture in the tetraploid line while they developed 16 days after culture initiation in the diploid line. The highest mean shoot regeneration percentage and mean shoot number per petiole was recorded as 69.99% and 20.23 in the tetraploid line while it was 45.57% and 12.61 in the diploid line (Table 7). Regenerated shoot length was again found to be higher in the tetraploid line 5 weeks after culture initiation. The mean shoot length was found as 2.8 cm in the tetraploid line, while it was 2.0 cm in the diploid line. The mean total shoot number per Petri dish, which can be determined by shoot regeneration percentage and shoot number per petiole, was recorded as 141.77 in the tetraploid line and 57.50 in the diploid line (Table 7).

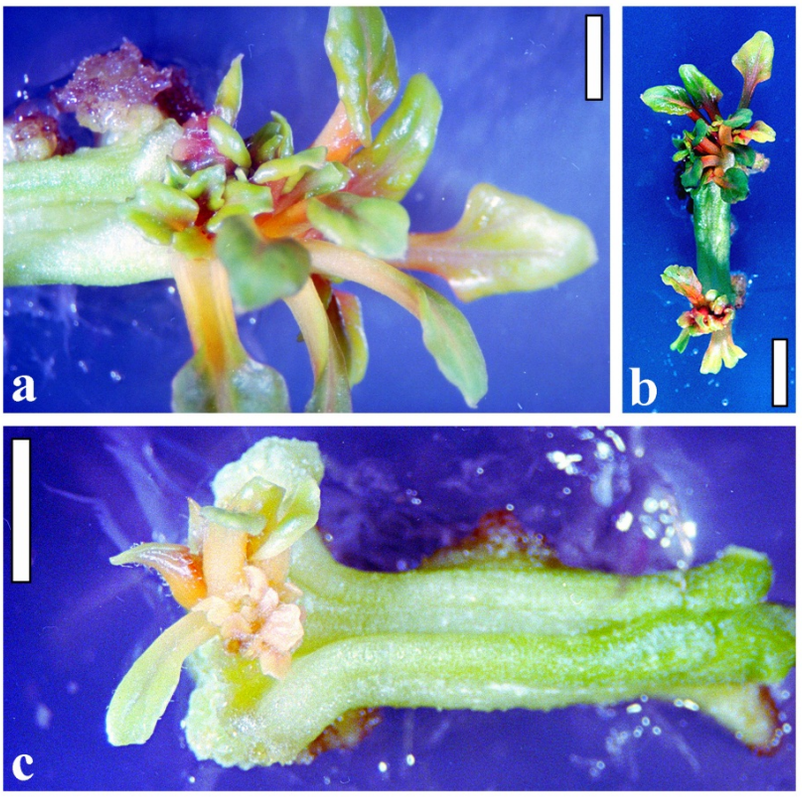

Figure 5. *In vitro* shoot regeneration from petiole explants of (a-b) 'CBM 315' (4X) and (c) 'ELK 345' (2X) line 5 weeks after culture initiation (Bars = 0.5 cm in a and c, 1 cm in b).

Shoots regenerated from petiole explants of the diploid and tetraploid lines were rooted on MS medium containing 3 mg l^{-1} IBA for 2 weeks. The best results were observed in shoots regenerated from petiole explants of the tetraploid line in all three experiments (Table 8). From the results, it is evident that shoots regenerated from petioles of the tetraploid line were more capable of establishing new plantlets than the ones grown from petioles of the diploid line (Figure 6a). Of the 70 shoots transferred to rooting medium, 61.3 shoots (87.62%) from tetraploid line 'CBM 315' and 51.7 shoots (73.81%) from the diploid line 'ELK 345' were rooted successfully (Table 8). Transferred plants reached harvest maturity in the field and no morphological abnormalities were observed.

4. *In vitro* susceptibility of genotypes to *Agrobacterium tumefaciens* infection at different ploidy levels

Agrobacterium-mediated transformation has been widely used for the introduction of foreign genes into plants and consequent regeneration of transgenic plants [44]. *A.tumefaciens* naturally infects the wound sites in dicotyledonous plants. Virulent strains of *A.tumefaciens*, when interacting with susceptible dicotyledonous plant cells, induce diseases known as crown gall

Figure 6. *In vitro* rooting and plantlet development from petiole explants of (a) 'CBM 315' (tetraploid) and (b) 'ELK 345' (diploid) line (Bar = 1.5 cm).

		Shoot Regeneration (%)		Shoot Number per Petiole		Shoot Length (cm)		Total Shoot Number per Petri Dish	
		'ELK 345' 2X	'CBM 315' 4X	'ELK 345' 2X	'CBM 315' 4X	'ELK 345' 2X	'CBM 315' 4X	'ELK 345' 2X	'CBM 315' 4X
1st experiment		40.0±5.773	66.7±3.333	12.53±0.617	22.80±1.106	2.2±0.577	3.2±0.153	50.12±9.181	152.73±14.589
	t value	3.957*		8.105**		6.124**		5.917**	
2nd experiment		46.7±3.333	70.0±5.774	13.70±0.656	19.70±0.929	1.9±0.153	2.7±0.058	63.67±3.844	138.97±12.305
	t value	3.368*		5.276**		3.703**		4.104**	
3rd experiment		50.0±5.774	73.3±3.333	11.60±0.625	18.20±0.557	1.8±0.100	2.5±0.115	58.70±9.650	133.60±8.426
	t value	3.501*		7.889**		4.583**		5.847**	
Mean[1]		45.57	69.99	12.61	20.23	2.0	2.8	57.50	141.77

Significantly different from zero at * $p < 0.05$ and ** $p < 0.01$

[1] Mean of three experiments

Table 7. Adventitious shoot regeneration from petiole explants of 'ELK 345' (diploid) and 'CBM 315' (tetraploid) 5 weeks after culture initiation on MS medium containing 1 mg l^{-1} BAP and 0.2 mg l^{-1} NAA

[45]. This strain contains a large megaplasmid (more than 200 kb), which plays a key role in tumor induction and for this reason it was named Ti (Tumor inducing) plasmid. The expression

		Number of Shoots Rooted		% of Shoots Rooted	
		'ELK 345' 2X	'CBM 315' 4X	'ELK 345' 2X	'CBM 315' 4X
1st experiment		53±1.155	64±1.000	75.71±1.648	91.43±1.430
	t value	7.201**		6.662**	
2nd experiment		52±1.528	59±1.155	74.28±2.181	84.29±1.648
	t value	3.656*		3.709*	
3rd experiment		50±1.528	61±1.399	71.43±2.181	87.14±1.648
	t value	5.745**		5.692**	
Mean[1]		51.7	61.3	73.81	87.62

Significantly different from zero at * $p < 0.05$ and ** $p < 0.01$

[1] Mean of three experiments

Table 8. *In vitro* root development of shoots regenerated from petiole explants of 'ELK 345' (2X) and 'CBM 315' (4X) lines on rooting medium enriched with 3 mg l^{-1} IBA 2 weeks after culture initiation.

of T-DNA genes of Ti-plasmid in plant cells causes the formation of tumors at the infection site. Two genetic components of bacteria, virulence genes (*vir*) and chromosomal genes (*chv*), are directly involved in the transfer of T-DNA from *Agrobacterium* to plant cells [44]. The molecular basis of *Agrobacterium*-mediated transformation is the transfer and stable integration of a DNA sequence (T-DNA) from the *Agrobacterium tumefaciens* Ti (tumor-inducing) plasmid into the plant genome leading to plant cell transformation [46, 47].

In a study conducted by Yildiz et al. [48], it was aimed to determine the susceptibility level of two sugar beet lines to wild-type *Agrobacterium tumefaciens* infection and ploidy effect on gene transfer efficiency under *in vitro* conditions. To evaluate the susceptibility of sugar beet lines at different ploidy levels against *Agrobacterium* infection, tumor formation was scored using the virulent strains 'A281' and 'A136NC'.

Among two lines used in the study, 'CBM 315' gave the highest results in three parameters studied in 'A281' wild strain. In 'CBM 315', tumor induction percentage, tumor diameter and number of tumors per explant were scored as 94%, 3.88 mm and 7.78, respectively (Figure 7). 'CBM 315' was followed by 'ELK 345' as 62% in tumor induction percentage, 1.80 mm in tumor diameter and 4.03 in number of tumors per explant (Table 9). In 'A136NC' wild strain, the highest values in tumor induction percentage, tumor diameter and number of tumors per explant were obtained from 'CBM 315' as 96%, 4.24 mm and 8.13 whereas lowest results were recorded from line 'ELK 345' as 73%, 2.14 mm and 4.36, respectively (Table 9).

The virulence of the bacterium depends on the strain and its interaction with the host plant. Various plant species differ greatly in their susceptibility to infection by *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* [49-53].

Even within a species, different cultivars or ecotypes may show vastly different degrees of susceptibility to tumorigenesis by particular *Agrobacterium* strains [54-56]. These differences

have been noted in rice [57], maize [58], various legumes [55], aspen [56], cucurbits [59], *Pinus* species [60], tomato [61], *Arabidopsis* [62], grape [63], and other species. Although some differences in transformation frequency may be attributed to environmental or physiological factors, a genetic basis for susceptibility has clearly been established in a few plant species [62, 64- 66].

Several researchers have reported that susceptibility to *Agrobacterium* transformation of various tissues, organs and cell types within a plant may differ. De Kathen and Jacobsen [67] reported that only dedifferentiating cells near the vascular system of cotyledon and epicotyl sections of *Pisum sativum* were susceptible to *Agrobacterium* transformation. Sangwan et al. [68] showed that only dedifferentiating mesophyll cells were competent for transformation in *Arabidopsis* cotyledon and leaf tissues.

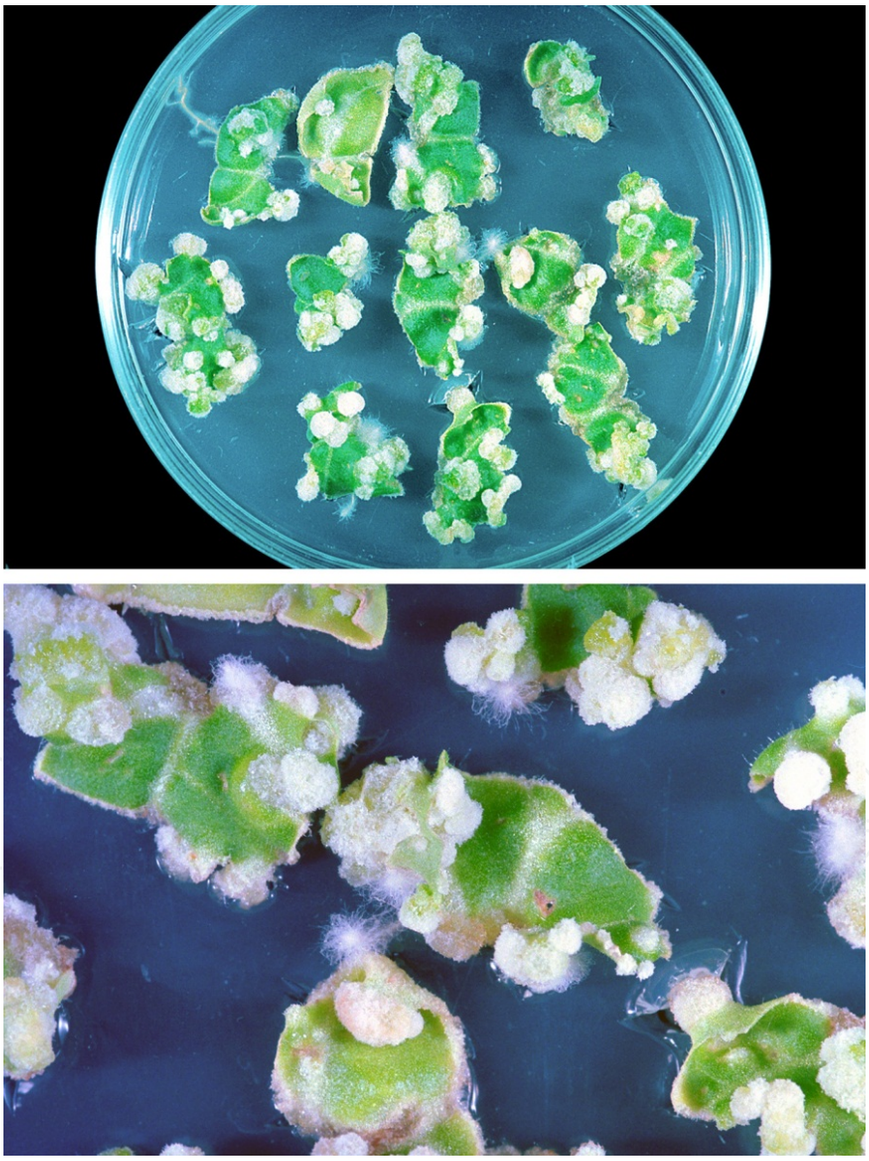

Figure 7. *In vitro* tumor formation caused by 'A281' virulent strain of *Agrobacterium tumefaciens* on leaf-disc explant of tetraploid sugar beet line 'CBM 315'

Genotype	A281			A136NC		
	Tumor Induction (%)	Tumor Diameter (mm)	No of Tumors / Explant	Tumor Induction (%)	Tumor Diameter (mm)	No of Tumors / Explant
'ELK 345' (2X)	62	1.80	4.03	73	2.14	4.36
'CBM 315' (4X)	94	3.88	7.78	96	4.24	8.13
t values	7.849**	5.881**	6.208**	3.994**	8.502**	7.808**

Each value is the mean of 3 replications with 12 explants

** Significantly different at the 0.01 level

Table 9. Response of two sugar beet lines at different ploidy levels to *Agrobacterium tumefaciens* virulent strains 'A281' and 'A136NC' 4 weeks after leaf-disc inoculation

The host-range limitation is perhaps the greatest disadvantage of *Agrobacterium*-mediated transformation although it is the most common used vector for the introduction of foreign genes to many crop plants, especially to dicotyledonous. The results were in accordance with the previous studies indicating strain and genotype differences [69-71].

From the results, it could be concluded that sugar beet lines have susceptibility to *Agrobacterium* infection with different levels. Moreover, if Table 9 was examined carefully, an interesting point came to attention that the difference in tumor induction might be related to ploidy level. Actually, in both strains of *Agrobacterium*, the highest results were obtained from 'CBM 315' which was tetraploid. Analysis showed that 'CBM 315' to be more beneficial for tumor induction and more susceptible to *Agrobacterium tumefaciens*.

It was reported that increased ploidy levels resulted in bigger cell size [72]. As it is known *Agrobacterium* infects cells at wound sites and size of the cells in this sites may influence transformation efficiency. The difference between diploid and tetrapoloid sugar beet lines with respect to wild-type *Agrobacterium tumefaciens* susceptibility might be related to ploidy levels. To our knowledge, this was the first report indicating that gene transfer efficiency might be affected from cell size at wound sites. However, this finding must be verified repeatedly by detealed studies.

5. Plant cellular response to salt stress at different ploidy level

The number of chlorophyll-containing chloroplasts increases from diploids to polyploids. Chlorophyll content and other proteins were shown to almost double from diploid to polyploid plants [40]. The cells with high ploidy level have bigger vacuoles and vacuole plays an important role in regulating osmotic pressure of the cell [38]. Higher cell osmotic pressure in polyploid plants cause to high tissue metabolic activity by increasing water and hormone uptake from the medium. Additionally, the increase in ploidy level leads to larger cell that has high growth rate. Polyploid genotypes have a higher water content and organic solutes than diploid

genotypes [42]. Chromosome number determines the size of leaves, the size of cells, the number of chloroplasts per cell and amounts of photosynthetic enzymes and pigments in cell [26]. As chromosome number increased, DNA content per cell, enzyme activity per cell, cell volume and photosynthesis per cell are all increased. In general, photosynthetic capacity of larger cells in polyploid plants is higher than smaller cells with lower chromosome numbers [5, 6, 43].

In a study conducted by Yildiz et al. [73], the responses of sugar beet genotypes at different ploidy levels to salt stress were evaluated. Diploid ('Felicita') and tetraploid ('AD 440') sugar beet genotypes were grown in pots, 1-month-old seedlings were treated with NaCl at different concentrations (0, 50 and 150 mM). Four days after NaCl application, cytological observations (the number of cell and stomata in the field of view area, lengths and widths of cells and stomatas) and 8 days after, seedling and root lengths were recorded.

Root lengths of both genotypes increased by increasing NaCl concentrations. Root length was recorded as 7.25 cm in diploid genotype 'Felicita' at 150 mM NaCl while it was 7.90 cm in tetraploid genotype 'AD 440'. Seedling lengths also increased by increasing NaCl concentration. Seedling length was the highest in diploid genotype as 11.25 cm while it was only 7.90 in tetraploid genotype (Table 10). Damages of increasing NaCl concentration were seen clearly in the leaves of seedlings. At higher NaCl concentrations, tissue necrosis was observed (Figure 8).

It was observed that cell number decreased by increasing NaCl concentration in both genotypes. However, decrease rate in cell number was higher in diploid genotype than tetraploid. This was most probably due to bigger cell size in tetraploid genotype and consequently there was few cells in the unit area. Lower cell number could be attributed to slow cell division as reported by Comai [10]. Cell length and width increased by increasing NaCl concentration. However, the highest values related to cell length and width were recorded in 150 mM NaCl concentration in diploid genotype as 40.28 μm and 29.14 μm while they were realized in 50 mM NaCl in tetraploid genotype 'AD 440' as 70.56 μm and 49.13 μm. In diploid genotype 'Felicita', approx. cell area was recorded as 652.59 μm^2 in control (0 mM NaCl) while it was 1173.75 μm^2 in 150 mM NaCl treatment. Approx. cell area was found almost two times more in diploid genotype when NaCl concentration was 150 mM. On the other hand, in tetraploid genotype 'AD 440', approx. cell area was found as 1372.14 μm^2 in 0 mM NaCl (control) treatment whereas it was 3466.61 μm^2 in 50 mM NaCl. The highest results in the parameters of cell length, cell width and approx. cell area were noted from 50 mM NaCl treatment in tetraploid genotype (Table 11).

Genotype	Root Length (cm)			Seedlings Length (cm)		
	0 mM NaCl	50 mM NaCl	150 mM NaCl	0 mM NaCl	50 mM NaCl	150 mM NaCl
'Felicita' (2X)	5.25 b	5.50 b	7.25 a	8.75 b	10.75 a	11.25 a
'AD 440' (4X)	6.05 b	6.83 ab	7.90 a	5.80 b	7.33 ab	7.90 b

Values followed by the different letters in a row are significantly different at the 0.01 level

Table 10. The effect of different concentrations of NaCl on sugar beet seedling development 8 days after salt treatment

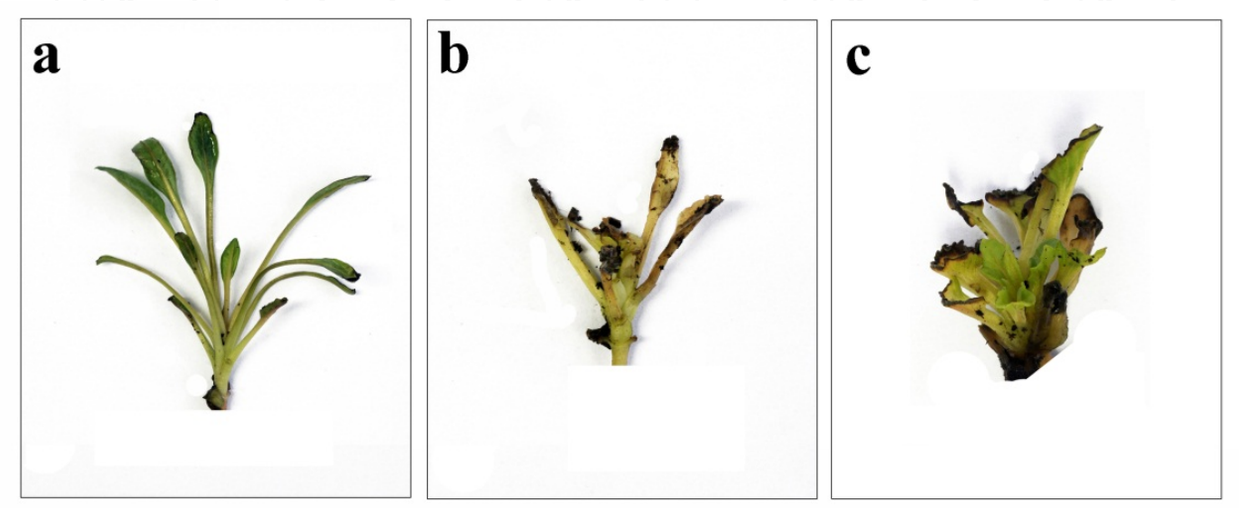

Figure 8. Sugar beet leaf development of cv. 'Felicita' 8 days after salt treatment (a) 0 mM NaCl (control), (b) 50 mM NaCl and (c) 150 mM NaCl

Number of stomata decreased by increasing NaCl concentration and this decrease was compensated by increased stomata size as reported by Inze and De Veylder [16]. Higher NaCl concentration increased stomata length and decreased stomata width in tetraploid genotype 'AD 440'. The highest stomata area was recorded from 150 mM NaCl treatment in diploid genotype while it was noted from 50 mM in tetraploid (Table 11).

The highest cell and stomata numbers were recorded from 0 mM NaCl treatment in both genotypes. And also cell and stomata numbers decreased by increasing NaCl concentration. However, this decrease in cell and stomata numbers were observed sharper in diploid genotype than in tetraploid one. The difference in cell and stomata numbers between 0 and 150 mM NaCl treatments was higher in diploid than in tetraploid. This could be due to the fact that tetraploid genotype 'AD 440' was more resistant to salt stress than diploid genotype 'Felicita' (Table 11). In other characters (cell and stomata lengths, cell and stomata widths, approx. cell and stomata areas), the highest values were recorded from 150 mM NaCl treatment in diploid genotype while they were noted from 50 mM NaCl treatment in tetraploid genotype. Since many characters in 150 mM NaCl concentration were almost the same as in 0 mM NaCl, it could be concluded that tetraploid genotype 'AD 440' was more resistant to salt stress than diploid one.

	Cell Number			Cell Length (μm)			Cell Width (μm)			Approx. Cell Area (μm²)		
Genotype	0 mM NaCl	50 mM NaCl	150 mM NaCl	0 mM NaCl	50 mM NaCl	150 mM NaCl	0 mM NaCl	50 mM NaCl	150 mM NaCl	0 mM NaCl	50 mM NaCl	150 mM NaCl
'Felicita' (2X)	162.70 a	124.50 b	70.70 c	31.71 b	27.14 b	40.28 a	20.58 b	19.28 b	29.14 a	652.59 b	523.25 b	1173.7 5 a
'AD 440' (4X)	84.60 a	52.40 c	69.30 b	54.58 b	70.56 a	44.85 b	25.14 b	49.13 a	30.85 b	1372.1 4 b	3466.6 1 a	1383.6 2 b
	Stomata Number			Stomata Length (μm)			Stomata Width (μm)			Approx. Stomata Area (μm²)		
Genotype	0 mM NaCl	50 mM NaCl	150 mM NaCl	0 mM NaCl	50 mM NaCl	150 mM NaCl	0 mM NaCl	50 mM NaCl	150 mM NaCl	0 mM NaCl	50 mM NaCl	150 mM NaCl
'Felicita' (2X)	18.80 a	18.20 b	7.30 c	26.28 b	21.42 b	28.57 a	18.85 b	18.85 b	20.56 a	495.37 b	440.39 c	538.54 a
'AD 440' (4X)	13.50 a	11.30 b	9.50 b	36.56 b	38.84 a	37.63 b	22.42 b	23.99 a	21.70 c	877.07 b	881.29 a	842.82 c

Values followed by the different letters in a row are significantly different at the 0.01 level

Table 11. Cellular responses to salt stress of sugar beet genotypes at different ploidy levels

6. Conclusion

Polyploidy is a common phenomenon in nature. There are differences between diploid and polyploid plants from morphological, physiological, cellular and biochemical aspects. Although polyploid genotypes have several advantages over diploids, the effects of increased ploidy level cannot be anticipated all the time. This was seen clearly in the studies we conducted. From one hand, diploid genotypes found superior than tetraploids in the generative characteristics such as total chlorophyll and protein contents, root and sugar yields, and sugar content under field conditions, on the other hand, regeneration capacity and susceptibility to *Agrobacterium tumefaciens* infection of polyploids were found higher under *in vtiro* conditions. Moreover, when cellular responses were examined, tetraploid genotype seemed more resistant to salt stress than diploid counterpart. Thus, it should be considered that responses of polyploid genotypes may differ from mophological, physiological, cellular and biochemical aspects. That is why, in a research study, responses of both diploid and polyploid genotypes should be evaluated carefully for successful results.

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