TÜBİTAK

Research Article

Turk J Bot
33 (2009) 383-387

doi:10.3906/bot-0811-10

Dynamics of 2,4-D in generation of cytomorphological variants in an important anticancerous and antihepatotoxic herb – *Cichorium intybus* L.

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Received: 20.11.2008
Accepted: 04.09.2009

Abstract: The genotoxic effect of 2,4-D was investigated in chicory (2n = 18). At 100 ppm concentration 4 variants differing in morphological traits were isolated and subjected to chromosomal analysis. Morphological variations in shape, height, and yield parameters were observed. Different cytological anomalies such as univalents, multivalents, bridges, laggards, chromosome stickiness, and polyads occurred in all 4 isolates. However, the frequency of these chromosomal irregularities decreased at anaphase, exhibiting recovery at later stages. Thus the above concentration of growth hormone 2,4-D can be effectively incorporated for raising viable mutants in this medicinally useful herb.

Key words: 2,4-D, *Cichorium intybus*

Introduction

Cichorium intybus L. is an important medicinal plant of family Asteraceae, believed to be a native of the temperate part of the Old World. It is grown mainly for medicinal purposes, as food or fodder or, as is more often the case, for the roots, which are commercially valuable. Chicory is an excellent mild bitter tonic for the liver and digestive tract; therefore, its extract forms an important constituent in liver medicines like Liv-52 and Geriforte. The cultivated plant is also used for curing diarrhoea, enlargement of the spleen, jaundice, fever, and vomiting. Juice of the plant is used in the treatment of uterine cancer and tumours. Leaves and roots are important for curing mouth, breast, and face cancer (Hartwell 1967-1971). Dried root powder is used for adulteration in coffee.

Conventional plant breeding approaches have exploited the available genetic variability in chicory, and as a result there has been a significant decline in genetic variability, which has led to a narrow genetic base of this crop. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement (Acharya et al., 2007). Traditional mutagenesis is the only method that can give rise to different mutant alleles with different degrees of trait modification.

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The mutagen 2,4-D (2,4-dichlorophenoxy acetic acid) used in the present investigation is a chlorinated phenoxy compound, widely used as a systemic herbicide, and also used in very low concentrations as a growth hormone in culture media. According to Amer and Ali (1974) it is an effective herbicide, induces chromosomal aberrations in meiotic cells of barley and wheat, and has produced heritable changes in awning, earliness, and stature. Therefore, the present study aimed to explore the possibilities of inducing beneficial alterations in the genotype to enhance genetic variability and also to observe the cytological behaviour of the variants isolated from the 2,4-D treated population.

Materials and methods

Fifty fresh and healthy seeds of *Cichorium intybus* were presoaked in distilled water for 12 h and then treated with 4 different concentrations (25, 50, 75, and 100 ppm) of 2,4-D prepared in phosphate buffer for 24 h with constant intermittent stirring. One set of seeds was soaked in distilled water to act as a control. The treated sets of seeds were washed in running tap water to remove the residual mutagen sticking to the surface of the seed coat. Each set was then sown in pots to raise M_1 generation. At 100 ppm only 25 out of 50 plants survived. For meiotic analysis young flower buds were selected from the 4 variants observed at 100 ppm of 2,4-D and also from the randomly selected control plants. The flower buds were fixed in Carnoy's fluid (alcohol:chloroform:acetic acid in a 6:3:1 ratio) for 40 min, transferred to propionic acid saturated with Ferric acetate for 24 h, and then stored in 70% alcohol. Anthers were squashed in propionocarmine (0.5%). Slides were made permanent in NBA series, mounted in Canada balsam and dried at 45 °C. More than 200 PMCs from each variant as well as the control were analysed for meiotic studies.

Observations

In the present investigation only 4 out of the 25 plants that survived at 100 ppm 2,4-D exhibited morphological variation. These 4 variants (variants 1, 2, 3, and 4) were found to be more vigorous than control and sibling plants of the same as well as different doses. These plants were differentiated on the basis of increased plant height, number of heads, number of seeds, and Tigrina type of chloromutant (Table 1).

To determine the fact that the variants observed in the present study were associated with point mutation or chromosomal changes, PMCs of the variants were observed and compared with control PMCs. In the control 215 PMCs were observed without any abnormality at any meiotic stage. At the diakinesis stage, 9 perfect ring bivalents were observed without any abnormality in the control (Figure 1A). PMCs of all the 4 variants isolated exhibited various chromosomal anomalies like univalent and multivalent (Figure 1B and 1C) at diakinesis, precocious separation, stray chromosome at metaphase, laggards, bridges (Figure 1D), and stickiness at anaphase, and polyads and bridges at telophase stages in varying frequencies; as a result percentage of abnormal PMCs ranged from 14.91% to 17.80% among the variants (Table 2).

Moreover, chiasma frequency at diakinesis as well as metaphase was also calculated and was found to be 16.12 at diakinesis and 15.32 at metaphase in the control (Table 2), while it decreased in all 4 observed variants at diakinesis as well as metaphase (Table 2). Pollen fertility seemed to be moderately affected, being 92.40%, 94.32%, 90.16%, and 92.83% in variants 1, 2, 3, and 4, respectively, as compared to the control, where it was 96.10% (Table 1).

Discussion

Induced mutagenesis has been accepted as a significant tool to break through the limitations of variability and to create variability in a short period of time (Yaqoob & Rashid, 2001; Akgun & Tosun, 2004; Kumar & Tripathi, 2007). The morphological changes that have been introduced in the variants through application of herbicide 2,4-D are due to various changes in the structure of chromosomes as varying degree of chromosomal anomalies at different meiotic stages have been observed during the present study. The correlation of chromosomal aberrations with morphological changes and other characteristics of plants has also been reported by various researchers in different plants, using various chemical mutagens (Grant, 1978; Kumar & Tripathi, 2007).

Chromosomal anomalies observed in the isolated variants have been considered as a reliable indicator of mutagenic activity (Mohandas and Grant, 1972). Occurrence of univalents in all 4 phenovariants may be due to induction of structural changes at the chromosome and gene level, which might be responsible for the failure of pairing among homologous chromosomes (Zeerak, 1992). Multivalent formation may be attributed to the partial homology between more than 2 chromosomes (Gaulden, 1987). Stickiness at prophase and metaphase appears as a result of improper folding of chromosome fibres (Klasterska et al., 1976) or due to partial dissociation and altered pattern of organisation of nucleoproteins (Myers et al., 1992) and depolymerisation of DNA causing increased affinity for stickiness. Precocious separation at metaphase stages resulted due to the disturbed spindle mechanism. Formation of a bridge at anaphase may be due to failure of chiasmata in a bivalent to terminalise and the chromosomes get stretched between the poles (Saylor & Smith, 1966). According to Sinha and Godward (1972), formation of bridges at the telophase stage may be due to paracentric

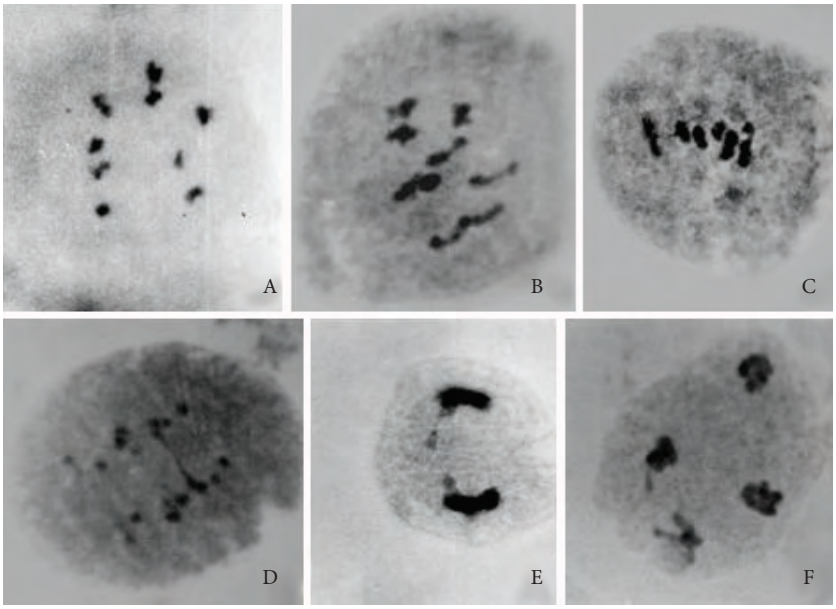

Figure 1. A: Prophase I: Diakinesis -9 perfect ring bivalents (control).
B: Diakinesis: 5 ring^{II} + 2 rod^{II} + 1^{IV}.
C: Metaphase-I: 3ring^{II} + 6 rod^{II} + 1^{IV}.
D: Anaphase-I: Chromatin bridge.
E: Telophase-I: Chromatin bridge.
F: Telophase-II: Broken Chromatin bridge.

Table 1. Effect of 2,4-D (100 ppm) on morphology, yield and pollen fertility in 4 isolated variants of chicory.

Treatments	Plant height heads/plant	Number of seeds/plant	Number of mutant type	Chlorophyll (%)	Pollen fertility
Control	115.46	140.00	2114.00	-	96.10
Variant 1	159.21	254.21	4064.21	-	92.40
Variant 2	148.11	235.46	3760.00	-	94.32
Variant 3	147.62	216.82	3456.00	Tigrina	90.16
Variant 4	164.36	205.61	3320.18	-	92.83

Table 2. Frequency of chromosomal aberrations in PMCs of 4 variants isolated from 2,4-D (100 ppm) treated population.

Control/ Variants	Total no. of PMCs	Diakinesis			Metaphase			Anaphase			Telophase		Abnormal PMCs
		Univalents	Multivalents	Chiasma freq.	Precocious separation	Stray chrom.	Chiasma freq.	Laggards	Bridges	Stickiness	Polyads	Bridges	
Control	215	_	_	16.12	_	_	15.32	–	–	–	–	–	–
Variant 1	222	1.35	1.80	15.43	3.60	3.15	14.02	2.25	0.93	2.70	0.90	0.45	17.11
Variant 2	219	0.91	2.73	15.84	2.73	2.73	13.23	3.65	0.81	1.82	1.36	0.91	17.80
Variant 3	228	0.43	2.19	14.38	2.19	1.31	14.48	3.07	1 .75	1.75	1.31	0.87	14.91
Variant 4	230	0.86	3.04	13.03	3.47	3.04	12.16	1.73	1.30	2.17	0.86	0.43	17.39

inversion. The laggard at anaphase may be due to a delay in terminalisation (Kumar & Tripathi, 2007) or failure of spindle fibres to bind on kinetochore. Laggards and non-oriented bivalents may produce micronuclei, if they fail to reach at poles in time to be included in the main nucleus, resulting in polyad formation in *Cichorium* sp. (Koduru & Rao, 1981). All these factors alone or together have resulted in the formation of defected microspores, which in turn lowered the pollen fertility, but the decrease was not significant enough to affect the yield.

Chlorophyll mutations, although not useful for plant breeding purposes, have been used as one of the measures of the mutagenic effect. Chlorophyll deficiency chimeras might have arisen from mitotic combination and gene conversion (Kunzel & Scholz, 1968). The increase in the mean values for the height and yield in mature plants may be due to the fact that 2,4-D in lower concentration also works as a growth regulator; stimulated auxin may also have changed the balance of endogenous growth regulators at the germination stage (Alizadeh et al., 2004) and thus affected the morphology. Increased yield of the mutagen induced variants may be due to the enhancing effect (Kothekar, 1983) and growth regulatory effect of mutagen (Audus, 1961). Significant increases in growth and yield in the present screened selected variants may be of great value from a breeder's point of view.

The above results suggest that a 100 ppm dose of the herbicide 2,4-D can be used to induce cytomorphological diversities in chicory for the selection of better mutants in further generations, because the more the variations the greater will be the chances for selection of better qualitative and quantitative characters.

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