Chromosome Structural Rearrangements in Onion (*Allium cepa* L.) through Induced Mutation

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Abstract: This study investigated whether there was any chromosomal structural rearrangement in individual chromosome of two mutant lines of onion viz., BP₂-100/1 and BP₂-100/2 compared to the parental chromosomes which may explain the seed to seed productions of the mutants in the same winter season even at late planting in January unlike the parent variety. The chromosome preparation was made from the root tip by haematoxylin method. No deviation in the number of chromosomes was found in the mutant lines but the length of the individual chromosomes was longer than that of their parent variety. Out of the eight pairs of homologous chromosomes, seven pairs were metacentric and one pair submetacentric in the parent “BARI Piaz-2”; five pairs were metacentric and three pairs submetacentric in BP₂-100/1; and three pairs were metacentric, three pairs submetacentric and two pairs of subtelocentric in BP₂-100/2. It was assumed that the length extension of the chromosomes and rearrangements in the arm ratio were due to gamma irradiation that induced duplication and or translocations of some regions of the chromosomes. Further research is needed to identify the induced genes and to establish relationships of the genes with the modified seed production behavior of the mutants.

Key words: Onion, mutation, chromosome, karyotype, idiogram.

1. Introduction

Onion (*Allium cepa* L.) belongs to the family Alliaceae, and is one of the most important and widely used vegetables and spices crops in many countries of the world. It is a diploid species with $2n = 2x = 16$ chromosomes [1]. In most of the countries of Asia, apart from its use as vegetable and salad, onion is generally used as a spice. It has great economic importance because of its medicinal and dietetic values. Among the spices, onion ranks first in Bangladesh in terms of production and area. Onion occupied an area of 126,175 ha with a total production of 1.051 million tons of bulb onions as against the estimated demand of 1.5 million tons [2]. This demand could be met in two ways, by increasing per

unit yield or by bringing more area under onion cultivation. Bringing more area under onion cultivation in winter is difficult although there is scope in the summer season. The existing summer varieties have low bulb and seed yield potentials. Mutation breeding technique is one of the important accessories to mainstream plant breeding. With this technique 15%-20% yield improvement and correction of the defects to a top cultivar are very easy and straight forward [3]. Pleiotropic effects are very common and help fix the breeding lines even in M₃/M₄ generations. Genetic improvement of any yield attributes either qualitative or quantitative in nature, has been successful with this technique [4-9]. Lagoda and Forster [10] confirmed the release of 3,218 crop varieties worldwide through this technique that also included some varieties of onion.

Plant Breeding Division of Bangladesh Institute of Nuclear Agriculture (BINA) has developed some

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mutants by irradiating the seeds of the summer onion variety “BARI Piaz-2” which have good seed setting ability, higher yield potential with longer shelf life. These mutants produce seed from seed in the same winter season and even at late planting in January unlike the parent [11]. The parent produces mostly bulb in the 1st year and seeds from those bulbs in the following year at optimum planting in October-November. For elucidating the underlying causes, this study was undertaken to investigate whether there was any chromosomal variation either in the length or in structure of the individual chromosome compared to the parental chromosomes which can explain the modified seed production behavior of the mutants.

2. Materials and Methods

2.1 Materials

Two mutant lines viz., BP₂-100/1, BP₂-100/2 along with the parent variety “BARI Piaz-2” were used in this study. These mutants were developed by irradiating the seeds of “BARI Piaz-2” with 100 Gy dose of gamma rays followed by selections

2.2 Collection of Root Tips and Fixation

Healthy and vigorously growing roots of 1.0-1.5 cm long of three genotypes were collected. Then the collected roots were thoroughly washed and pretreated in saturated aqueous solution of L-monobromonaphthalene (MBN) for 2.5 h with occasional stirring. After washing, the pre-treated roots were fixed in freshly prepared acetic alcohol (1:3).

2.3 Staining of Roots for Cytological Study

Fixed roots were thoroughly washed and these were hydrolysed in small vials with 10% HCl at 60 °C in an oven for 3-4 min. After hydrolysis, the roots were then treated with 2% aqueous solution of iron alum (ferric ammonium sulphate) for 3-4 min for mordanting. Iron-alum treated roots were then washed thoroughly.

The roots were then stained in 0.5% haematoxylin solution for 3-4 min and washed for several times until the coloring of the water stopped.

2.4 Slide Preparation

For slide preparation, the stained root was kept on a clean slide. The meristematic tip portion of about 1.0-1.5 mm was excised and the remaining back portion of the root was thrown away. The excised tip portion was squashed in 0.25%-0.5% aceto-carmine solution after putting the cover glass. The slide was then dried over a flame, followed by repeated heat-cool-press process.

2.5 Photo Micrographic Study

Photomicrography of the chromosome plates was done from slides. Photomicrographs of the selected chromosome plates were taken with the aid of Olympus Research Microscope Model BX40 using plan 100× objective. Sony Cyber Shot 16 megapixel DX Film was used. Chromosome measurements were made from 10 cm × 16 cm photo prints of chromosome plates and chromosome length was measured in millimeter (mm) based on calculated print magnification. The magnification of microscope, eye piece, tunnel and print magnifications were 100×, 40×, 10× and 1,600×, respectively.

2.6 Karyotype Study

Three representative prometaphase plates of two mutant lines, BP₂-100/1 and BP₂-100/2, and the parent variety “BARI Piaz-2”, were selected for karyotypic observation. A scatter diagram for each selected cell was prepared using total lengths and arm ratio of the 16 chromosomes of the complements. Each chromosome and its corresponding points on the diagram were numbered. The chromosomes were then paired by circling the corresponding points on the scatter diagram based on the proximity of the two points. Thus, the eight pairs of points in the scatter diagram were determined which represent eight pairs

of homologous chromosome complements. The average total length and arm ratio of each of the eight pairs of chromosomes constituted the haploid complement of that cell.

3. Results

3.1 Somatic Chromosome Number and Morphology

The somatic chromosome number of the parent variety “BARI Piaz-2” and two mutant lines BP₂-100/1 and BP₂-100/2 were $2n = 16$ (Figs. 1a-1c). No deviation in chromosome number was found in microscopic observation from the root tip. In the prometaphase plates, fewer dividing cells and longer chromosomes were observed in the mutant lines than that in the parent variety. Secondary constriction in one chromosome was detected in both the mutant lines and the parent variety despite its location varied between the parent and the mutants. No satellite chromosome was visible during this preparation.

3.2 Chromosome Length

Scatter diagrams prepared from the photographic measurement of chromosomes of the representative

plates for “BARI Piaz-2”, BP₂-100/1 and BP₂-100/2 are shown in Figs. 2-4. The magnified total length of individual chromosome ranged between 15.18 mm and 34.29 mm and the arm ratio 1.01 to 2.36 in “BARI Piaz-2” (Table 1). In contrast, total length of individual chromosome ranged between 26.64 mm and 37.75 mm and the arm ratio 1.05 to 2.59 in the mutant BP₂-100/1 (Table 2). Total length of individual chromosome of the other mutant BP₂-100/2 ranged between 17.64 mm and 36.26 mm and arm ratio 1.14 to 3.72 (Table 3).

3.3 Karyotype

Chromosome type was assessed following the classification system of Levan and Sandberg [12] and others. Based on the arm ratio “BARI Piaz-2” showed seven pairs of metacentric and one pair of submetacentric chromosomes (Table 4) while the mutant BP₂-100/1 showed five pairs of metacentric and three pairs of submetacentric chromosomes (Table 5) and the other mutant BP₂-100/2 showed three pairs of metacentric and three pairs of submetacentric and two pairs of subtelocentric chromosomes (Table 6).

Table 1 Measurement of the long arm, short arm and arm ratio of the chromosomes in representative plate of the cell of the parent variety “BARI Piaz-2”.

Chromosome number	Long arm (mm)	Short arm (mm)	Arm ratio	Total length (mm)
1	19.20	15.09	1.27	34.29
2	15.66	13.00	1.21	28.66
3	16.60	11.00	1.51	27.60
4	15.10	12.10	1.25	27.20
5	16.20	10.00	1.62	26.20
6	12.36	12.30	1.01	24.66
7	13.60	10.66	1.28	24.26
8	12.09	12.06	1.01	24.15
9	12.40	11.60	1.07	24.00
10	15.30	7.20	2.13	22.50
11	13.20	8.40	1.57	21.60
12	10.99	10.00	1.10	20.99
13	10.90	9.00	1.21	19.90
14	10.60	7.45	1.42	18.05
15	8.77	8.60	1.10	17.37
16	10.66	4.52	2.36	15.18
Mean $\pm$ SE	13.35 $\pm$ 0.70	10.19 $\pm$ 0.65	1.38 $\pm$ 0.10	23.54 $\pm$ 1.20

Fig. 1 Representative somatic chromosome complements in roots of the onion parent variety “BARI Piaz-2” (a), mutant lines, BP₂-100/1 (b) and BP₂-100/2 (c).

Arrow indicates secondary constriction.

Fig. 2 Scatter diagram of the representative plate of the cell of the parent variety “BARI Piaz-2”.

Each pair of points circled is considered representing a homologous pair.

Fig. 3 Scatter diagram of the representative plate of the cell of BP₂-100/1 mutant line.

Each pair of points circled is considered representing a homologous pair.

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Fig. 4 Scatter diagram of the representative plate of the cell of BP₂-100/2 mutant line.

Each pair of points circled is considered representing a homologous pair.

Table 2 Measurement of the long arm, short arm and arm ratio of the chromosomes in representative plate of the cell of the mutant BP₂-100/1.

Chromosome number	Long arm (mm)	Short arm (mm)	Arm ratio	Total length (mm)
1	20.10	17.65	1.14	37.75
2	19.70	17.22	1.14	36.92
3	18.84	16.70	1.13	35.54
4	18.36	16.69	1.10	35.05
5	24.58	10.45	2.35	35.03
6	23.32	11.67	1.99	34.99
7	24.96	9.65	2.59	34.61
8	18.75	15.39	1.22	34.14
9	18.75	11.87	1.58	30.62
10	16.63	11.72	1.42	28.35
11	17.70	16.81	1.05	28.03
12	17.59	10.33	1.70	27.92
13	14.36	13.20	1.09	27.56
14	17.40	9.67	1.80	27.07
15	18.84	7.80	2.42	26.64
16	14.56	6.00	2.43	20.56
Mean $\pm$ SE	19.03 $\pm$ 0.77	12.68 $\pm$ 0.91	1.63 $\pm$ 0.14	31.30 $\pm$ 1.21

Table 3 Measurement of the long arm, short arm and arm ratio of the chromosomes in representative plate of the cell of the mutant BP₂-100/2.

Chromosome number	Long arm (mm)	Short arm (mm)	Arm ratio	Total length (mm)
1	24.46	11.80	2.07	36.26
2	20.54	8.36	1.66	32.92
3	21.46	12.38	2.51	30.02
4	19.72	7.26	2.36	28.08
5	18.83	4.28	2.02	28.16
6	15.60	8.50	1.35	27.16
7	21.30	5.73	3.72	27.03

(Table 3 continued)

Chromosome number	Long arm (mm)	Short arm (mm)	Arm ratio	Total length (mm)
8	14.81	4.88	1.29	26.25
9	16.87	11.44	2.96	22.57
10	13.79	5.70	1.62	22.29
11	15.55	9.33	3.17	20.45
12	13.86	4.90	2.84	18.74
13	10.83	8.26	1.42	18.45
14	9.38	8.56	1.14	17.64
15	11.75	7.62	2.75	16.03
16	10.89	3.72	1.50	18.15
Mean $\pm$ SE	16.23 $\pm$ 1.11	7.67 $\pm$ 0.67	2.15 $\pm$ 0.19	24.39 $\pm$ 1.49

Table 4 Morphological features of the proposed standard karyotype in onion variety “BARI Piaz-2”.

Chromosome number	Name of identified chromosome	Total length (mm)	Arm ratio	Chromosome type
1	m1	31.48	1.24	m [*]
2	m2	26.90	1.57	m
3	m3	25.73	1.27	m
4	m4	24.41	1.01	m
5	m5	22.50	1.09	m
6	m6	19.81	1.50	m
7	m7	18.63	1.16	m
8	sm1	18.84	2.25	sm ⁺

*The letter m represents metacentric and ⁺sm submetacentric chromosome.

Table 5 Morphological features of the proposed standard karyotype in mutant onion line BP₂-100/1.

Chromosome number	Name of identified chromosome	Total length (mm)	Arm ratio	Chromosome type
1	m1	37.34	1.14	m [*]
2	m2	35.30	1.12	m
3	m3	34.57	1.61	m
4	m4	29.49	1.50	m
5	m5	27.80	1.07	m
6	sm1	34.82	2.47	sm ⁺
7	sm2	27.50	1.75	sm
8	sm3	23.60	2.43	sm

*The letter m represents metacentric and ⁺sm submetacentric chromosome.

Table 6 Morphological features of the proposed standard karyotype in mutant onion line BP₂-100/2.

Chromosome number	Name of identified chromosome	Total length (mm)	Arm ratio	Chromosome type
1	m1	26.71	1.32	m [*]
2	m2	20.22	1.56	m
3	m3	18.05	1.28	m
4	sm1	34.59	1.87	sm ⁺

(Table 6 continued)

Chromosome number	Name of identified chromosome	Total length (mm)	Arm ratio	Chromosome type
5	sm2	28.12	2.19	sm
6	sm3	17.39	2.80	sm
7	st1	28.53	3.12	st ⁺⁺
8	st2	21.51	3.07	st

*The letter m represents metacentric, ⁺sm submetacentric and ⁺⁺st subtelocentric chromosome.

Fig. 5 Representative idiogram of the eight pairs of chromosomes in “BARI Piaz-2”.

The letter m represents metacentric and sm submetacentric chromosome.

4. Discussion

The cytological investigation of the parent variety “BARI Piaz-2” and two mutant lines, BP₂-100/1 and BP₂-100/2, revealed the presence of $2n = 16$ chromosomes in the root tip. No deviation in the $2n$ number of chromosome was observed. Lower number of dividing cells was observed in the mutant lines than that in the parent variety “BARI Piaz-2” during the slide preparation which was similar to the findings of Datta *et al.* [13]. The chromosomes of the mutant lines BP₂-100/1 and BP₂-100/2 were considerably longer than the parent variety “BARI Piaz-2”, and this might be due to duplication of some region of the chromosomes by the mutagenic effect of gamma ray. This result is supported by the observation in some other studies. Ferdoush [11] and Bolon *et al.* [14] also reported duplication of DNA in all the chromosomes of fast neutron irradiated population of soybean.

Though, onion has eight metacentric or submetacentric and one subtelocentric chromosomes with the nucleolus organizer region [15] but in this study, it was observed that the parent variety “BARI Piaz-2” had seven pairs of metacentric and one pair of submetacentric chromosomes and no subtelocentric chromosome (Fig. 5). In contrast, in the mutant BP₂-100/1, there were five pairs of metacentric and three pairs of submetacentric chromosomes (Fig. 6) and the other mutant BP₂-100/2 had three pairs of metacentric, three pairs of submetacentric and two pairs of subtelocentric chromosomes (Fig. 7). The changes in the structure of the chromosomes of the two mutants compared to the parent were assumed to be the effect of gamma ray (ionizing radiation). It is generally and widely accepted that ionizing radiation is reported to induce structural rearrangements in plant genomes through deletion [16], duplication [17], inversion and translocation [18].

Fig. 6 Representative idiogram of the eight pairs of chromosomes in BP₂-100/1.

The letter m represents metacentric and sm submetacentric chromosome.

Fig. 7 Representative idiogram of the eight pairs of chromosomes in BP₂-100/2.

The letter m represents metacentric, sm submetacentric and st subtelocentric chromosome.

5. Conclusions

It may be concluded that the modified seed production behavior of the two mutants, i.e., production of seed from seed in the same winter

season even at late planting in January unlike the parent variety, might be due to the gamma ray which induced duplication and or translocations of some regions of the chromosomes of the mutants. The

predicted gene dose effects might have helped modification of seed production behavior of the mutants that needs further investigation to identify the induced genes and to establish relationships of the genes with the modified seed production behavior of the mutants.

Acknowledgments

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