Indian J. Genet., **60**(4): 451-458 (2000)

CYTOMORPHOLOGICAL STUDIES OF TRISOMICS ISOLATED FROM DESYNAPTIC MUTANTS IN BARLEY (*HORDEUM VULGARE* L.)

M. R. SINGH AND GANESH PRASAD*

Department of Agricultural Botany, P.G. College, Ghazipur 233 001

(Received: October, 1999; accepted: August, 2000)

ABSTRACT

From the selfed progency of three desynaptic mutants, 38 primary trisomic plants were isolated. These trisomic plants were classified into seven types based on their identifiable morphological traits. Pollen sterility ranged from 35-90 per cent and ovule sterility ranged from 56-97 per cent. They showed different types of chromosome associations at MI which were trivalent, bivalent and univalent. Range of univalents was 2-13 while, the range of bivalents was 1-6 per cell. Average number of trivalents/cell was 0.09 to 0.43, average number of bivalents/cell was 3.3-4.16 and average number of univalents/cell was 5.93-7.41 in these trisomics. Different types of AI separations such as 9-6 and 8-7 were observed and the average number of laggards/cell was 0.96-1.4. Lagging of chromosomes and formation of bridges at A II and presence of micronuclei in spores were also observed in these trisomics.

Key Words : Barley, desynaptic mutants, trisomics, cytomorphology

Desynapsis has been reported in many plant species [1-7]. Soost[8] and Burnham[9] suggested the use of asynaptic and desynaptic genes to induce aneuploids. Using the desynaptic genes Gottschalk and Milutinovic [10] isolated trisomics from the progeny of desynaptic mutants of *Pisum*. The present paper deals with the cytomorphological studies of trisomics obtained in the progeny of desynaptic mutants of barley.

MATERIALS AND METHODS

Abnormal looking plants were isolated from the selfed progeny of three desynaptic mutants, namely DM6-6, DM16-7 and DM 1-15. Anthers from these plants were collected in 3:1 alcohol-acetic acid solution and preserved in 70 percent ethanol. The slides were prepared in 2 percent aceto-carmine. Meiotic study showed that some of the abnormal looking plants were trisomics. Data on morphological characters were recorded and the trisomics were identified according to Tsuchiya [11, 12].

*Dept. of Agricultural Botany, S.D.J. P.G. College, Chandesar, Azamgarh, 276 128

RESULTS AND DISCUSSION

From the selfed progeny of desynaptic mutants a total of 38 trisomic plants were obtained. The average frequency of their occurrence was 6.6 per cent (Table 1). Unequal separations are often observed in desynaptic and asynaptic mutants [1, 3, 7, 10, 13]. It leads to the formation of gametes containing an extra chromosome (n + 1) which after fertilization with normal gamete(n) produce trisomics (2n + 1). Trisomics have been isolated from the progeny of desynaptic mutants in some crop species [10, 13]. Isolation of present trisomics supports the findings of above workers.

Table 1. Frequencies of trisomics isolated from the selfed progeny of desynaptic mutants

Desynaptic mutant	Population size	Types of trisomics and their frequencies								
		Triplo 1	Triplo 2	Triplo 3	Triplo 4	Triplo 5	Triplo 6	Triplo 7	Total	Trisomic (%)
DM 6-6	207	1	3	-	-	3	2	4	13	6.3
DM 16-7	180	4	1	2	5	-	-	2	14	7.8
DM 1-15	185	-	5	3	2	-	1	-	11	5.9
Total	572	5	9	5	7	3	3	6	38	6.6

Trisomics may be distinguished by the characteristics of grains as well as other morphological features of the plants [14, 15]. All the trisomics varied from the normal diploid as well as from their desynaptic parents regarding morphological traits. They showed certain reduced identifiable morphological traits. On the basis of their gross morphology they were classified into seven different types (Tables 1, 2)

(i) **Triplo 1** (Bush) : The plants were semidwarf with many thick tillers. They had dark green, broad and normal leaves in length which were either bent or erect. The leaf index was 15.8 (Table 2). The plants had short and lax ears with comparatively smaller awns and shrivelled seeds.

(ii) **Triplo 2** (Slender) : Stems were slender, weak and thin, semi-erect with low number of tillers. The leaves were dark green, very long, narrow with smooth surface. Leaf index was 24.8. The spikes were short, lax with long and thin awns. The grains were small thin and light in weight.

(iii) **Triplo 3** (Pale) : The plants were weak, thin, semi-dwarf with few tillers. They contained light green, long, narrow and smooth leaves. Leaf index was 21.4. The spikes were weak and deformed with spreading long awns.

Table 2. Morphological traits, sterility and seed set in trisomics and their parents

Plant	Chromo-somes	Plant height (cm)	No. of tillers	Length of spike (cm)	Leaf index* L : B (L/B)	Sterility and seed set (%)		
						P	O	S
Normal	-	120.0	36	22.5	36.6:1.9(16.1)	1.3	2.4	97.60
Triplo 1	1	50.5	21	10.3	20.5:1.3(15.8)	38.5	56.5	43.5
Triplo 2	2	66.5	7	10.2	29.8:1.2(24.8)	35.4	80.5	19.5
Triplo 3	3	65.4	6	18.5	30.1:1.4(21.4)	42.1	63.5	36.5
Triplo 4	4	77.0	19	20.0	24.4:1.6(15.3)	40.7	89.5	10.5
Triplo 5	5	84.0	25	16.5	32.8:1.8(18.2)	56.6	94.4	5.60
Triplo 6	6	80.1	5	19.5	19.6:1.5(13.1)	41.5	79.5	20.5
Triplo 7	7	48.3	22	10.5	27.8:1.3(21.4)	89.9	97.5	2.50
DM 6-6	-	110.2	21	19.0	24.5:1.9(12.9)	30.0	31.5	68.5
DM 16-7	-	119.5	31	18.2	26.0:1.8(14.4)	33.7	35.5	64.50
DM 1-15	-	116.0	24	24.0	28.0:2.2(12.7)	39.3	34.6	65.4

*2nd leaf from top, L = Length (cm), B = Breadth (cm), L/B = Index, P = Pollen, O = Ovule, S = Seed set.

(iv) ***Triplo* 4** (Robust) : These plants were vigourous, dwarf to semi-dwarf and contained many thick tillers. The leaves were broad, dark green, short, thick, rough, erect with wavy margin. Leaf index was 15.3. Spikes were long with comparatively shorter awns. Seed were large and bold.

(v) ***Triplo* 5** (Pseudo-normal) : The plants were semi-dwarf with medium thick stems and produced many tillers. The leaves were long, broad, drooping and dark green. Leaf index was 18.2. The spikes were short, compact with thin awns. The grains were small, thin shrivelled and light.

(vi) ***Triplo* 6** (Purple) : The plants were erect and semi-dwarf with few tillers. The leaves were green, short, thick, rough and broad. Leaf index was 13.1. Spikes were short lax with long and coarse awns and long seeds.

(vii) ***Triplo* 7** (Semi-errect) : The plants were dwarf semi-erect with many thin tillers. The stems were thin, short, weak and contained short, erect and narrow leaves. Leaf index was 21.4. The spikes were short compact with medium awns. The seeds were long, thin and light.

Sterility

Sterility varied in different trisomics. Pollen sterility ranged from 35.4% (*Triplo* 2) to 89.9% (*Triplo* 7) in trisomics in contrast to 1.3% in normal, while in desynaptic mutants it was 30.0% (DM 6-6), 39.3% (DM 1-15) and 33.0% (DM 16-7) (Table 2). The ovule sterility was greater than pollen sterility. It varied from 56.5% (*Triplo* 1) to 97.5% (*Triplo* 7) in trisomics. Tsuchiya[11] found 2.8-27.7 per cent pollen sterility in trisomics derived from a two rowed barley. High sterility in the present trisomics was due to action of desynaptic genes present in the complement as well as the addition of an extra chromosome. Addition of an extra-chromosome to a complement results in reduced plant vigour, pollen and ovule sterility. It is due to change in the number of a particular chromosome which resulted in disbalance of genetic regulation. Each chromosome has separate effect according to its genetic content[11, 12, 16]. The morphological traits are generally used to identify the different types of trisomics [11, 17, 18]. To some extent, the trisomics reported here varied in morphological traits from those reported by earlier workers. This variation was due to varietal differences. These findings are consistent with those reported by Das and Srivastava [19], Das and Bhowmik [20], Das and Kundu [21], Prasad and Das [22] and Das and Prasad [16].

MEIOTIC OBSERVATIONS

(i) Prophase I : Bivalents and univalents were observed at diplotene and diakinesis. The extra chromosome of trisomic was seen as univalent or it formed a trivalent.

(ii) Metaphase I : Seven bivalents (7^{II}) were always seen in normal diploid (Fig. 1A), while the desynaptic mutants exhibited various types of configurations at MI, the most frequent configurations were $4^{II} + 6^{I}$ (70.96%) in DM 6-6, $4^{II} + 6^{I}$ (44.8%) in DM 1-15 and $5^{II} + 4^{I}$ (53.69%) in DM 16-7. The trisomics showed either "bivalents + univalents" (Fig. 1B, D, E, F) or bivalents + univalents + one trivalent" (Fig. 1C, G, H) in varying frequencies.

The range of bivalent was 1 to 6 in *Triplo* 6, 1 to 5 in *Triplo* 4, 2 to 5 in *Triplo* 1, 5 and 7 and 3 to 5 in *Triplo* 2 and 3. The univalents ranged from 2 to 13 in *Triplo* 4, 2 to 11 in *Triplo* 1 and 6 and ≥ to 9 in *Triplo* 2, 3, 5 and 7. The most frequent configurations were $4^{II} + 7^{I}$ in *Triplo* 1, 2, 3, 5 and 7 and $3^{II} + 9^{I}$ in *Triplo* 4 and 6. Frequency of the cells having one trivalent was the highest in *Triplo* 7 (42.9%) following *Triplo* 4 (32.7%), *Triplo* 6 (32.2%), *Triplo* 5 (24.4%), *Triplo* 1 (18.2%), *Triplo* 3 (11.3%) and *Triplo* 2 (8.8%).

Average number of trivalents per cell was the highest in *Triplo* 7 (0.43) followed by *Triplo* 4(0.33), while the average number of bivalents per cell was the highest in

Triplo 5 (4.16) followed by *Triplo* 3 (4.14) (Table 3). Average number of univalent per cell was the maximum in *Triplo* 4 (7.41) followed by *Triplo* 6 (6.82), while it was minimum in *Triplo* 5 (5.93).

Bivalents and trivalent were found arranged at or near the equator but univalents were always scattered towards the periphery of the cells. This tendency was observed in all the seven types.

The cytological configurations observed in seven trisomics at MI reveal the formation of bivalents, univalents and a trivalent. In normal cases the trisomics show $7^{II} + 1^{I}$, $6^{II} + 1^{III}$ or $6^{II} + 3^{I}$[23]. The formation of more univalents and less bivalents is due to the action of desynaptic genes in the complement causing failure of chiasma formation. Tsuchiya[23] observed 15^{I} at diakinesis and MI in about 1% PMC's of all slender trisomics. On this finding he concluded that asynaptic phenomenon may be ascribed to specific effect of chromosome 2. Formation of few bivalents and more univalents resembles to the desynaptic parents to some extent. The tendency of univalents to scatter towards the periphery agrees with those observed in desynaptic mutants [1, 7, 24, 25].

(iii) Anaphase I : Trisomics showed various types of chromosome separations at AI. The most frequent separation was 7-1-7 in all the trisomics. Other types of separation were 8-7 (Fig. 1J), 6-3-6 (Fig. 1 I) 8-1-6, 9-6, 7-2-6, 8-2-5, 7-3-5 and 6-4-5 which occurred in varying frequencies. The highest number of lagging chromosomes was 1.40/cell which was observed in *Triplo* 4. *Triplo* 6 showed the lowest number of laggards (0.96/cell) (Table 3). In some cases laggards showed precocious division

Table 3. Meiotic configurations at MI and AI in trisomics

Trisomics	Chromosome association at MI/cell				AI segregation			
	No. of PMC's examined	III	II	I	No. of PMC's examined	9-6 (%)	8-7 (%)	Laggards/ cell
Triplo 1	226	0.18	3.87	6.72	62	8.06	14.51	1.26
Triplo 2	193	0.09	4.01	6.71	98	17.35	12.24	1.22
Triplo 3	292	0.11	4.14	6.39	110	11.82	20.00	1.05
Triplo 4	346	0.33	3.30	7.41	120	10.00	16.67	1.40
Triplo 5	364	0.25	4.16	5.93	105	13.33	10.48	1.30
Triplo 6	205	0.32	3.60	6.82	108	11.11	25.06	0.96
Triplo 7	212	0.43	3.87	5.97	88	9.09	14.77	1.33

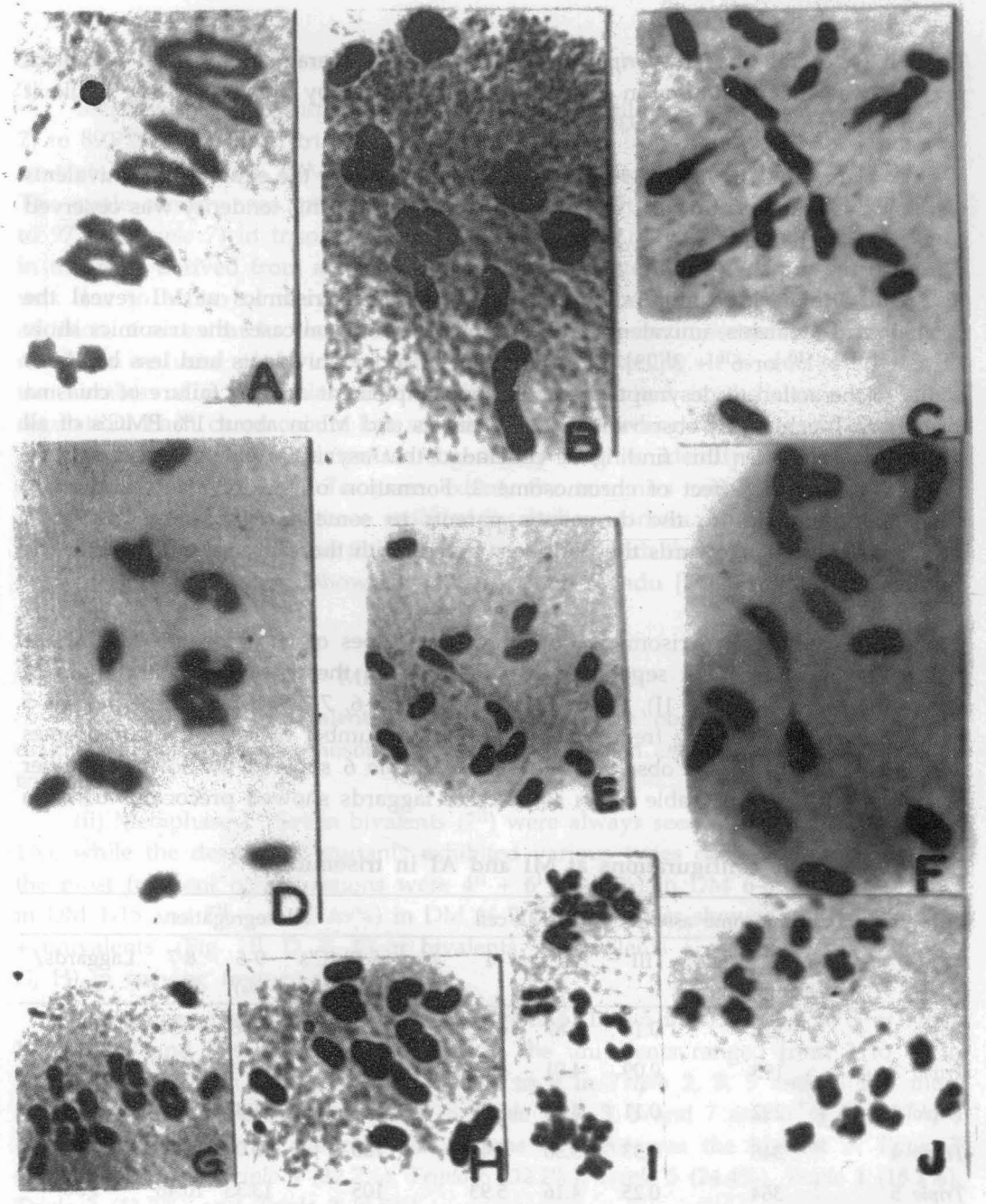

Fig. 1. A-7^{II} at MI in normal diploid. B-H-MI configurations in primary trisomics, B- $6^{II}+3^{I}$, C-$3^{II}+1^{III}+6^{I}$, D-$5^{II}+5^{I}$, E-$1^{II}+13^{I}$, F-$3^{II}+9^{I}$, G-$5^{II}+1^{III}+2^{I}$, H-$2^{II}+1^{III}+8^{I}$, I-J- AI configurations in primary trisomics, I-6-3-6 separation showing precocious centromere division in lagging chromosomes, J-8-7 separation.

of centromere (Fig. 1I). As far as more than one laggards are concerned it was thought to be due to the tendency of univalents to remain scattered towards the periphery of the cell and failure in proper orientation at equator. Presence of more univalents resulted in more laggards and unequal separation. In case of single univalent the extra chromosome always either lagged to result in 7-1-7 separation or it moved to one pole resulting 8-7 separation.

(iv) Other abnormalities : Many other meiotic abnormalities were also observed at A II and in spore quartet. The main abnormality at A II was lagging of chromosomes which ranged from 1 to 4 per cell. Laggards were present in one or both the cells of dyad. The trisomics obtained from DM 6-6 showed one laggard in both the cells whereas those obtained from DM 16-7 and DM 1-15 exhibited 1 to 4 laggards in either or both the cells of dyad. Occasionally few bridges were also observed in all types. The trisomics showed micronuclei in some cells of the quartet; the frequency varied from 1-3 per cell. Presence of one micronucleus was most frequent.

Abnormal chromosome separation and formation of micropollen resulted in high sterility. As discussed above the trisomics contain desynaptic genes in their complement hence they also exhibited the characters of desynaptic mutants. A high frequency of micronuclei in spores was observed in this study. Lagging of chromosomes resulted in micronuclei formation in spores. Prasad and Tripathi[7] in barley, Beasley and Brown[26] in *Gossypium* and Celarier[27] in *Tradescantia* have reported the formation of micropollens and occurrence of micronuclei containing sporse in asynaptic and desynaptic mutants.

REFERENCES

1. J. Sjödin. 1970. Induced asynaptic mutant in *Vicia faba* L. Hereditas., **66**: 125-232.
2. R. Riley and C. N. Law. 1965. Genetic variation in chromosome pairing. Adv. Genet., **13**: 57-114.
3. W. Gottschalk and S. H. Baquar. 1971. Desynapsis in *Pisum sativum* induced through gene mutation. Can. J. Genet. Cytol., **13**: 138-143.
4. R. G. Palmer. 1974. A desynaptic mutant in soybean. J. Hered., **60**: 280-286.
5. P. P. Jauhar and U. Singh. 1969. Desynapsis and blockage of meiosis in *Pennisetum orientale* Rich. Theor. Appl. Genet., **39**: 315-319.
6. R. G. Sahu, V. Tiwari and R. B. Singh. 1981. Induced desynapsis in safflower (*Carthamus tinctorius* L.). J. Cytol. Genet., **16**: 71-75.
7. G. Prasad and D. K. Tripathi. 1986. Asynaptic and desynaptic mutants in barley. Cytologia., **51**: 11-19.
8. R. K. Soost. 1951. Comparative cytology and genetics of asynaptic mutants in *Lycopersicon esculentum* Mill. Genetics., **36**: 410-434.

9. C. R. Burnham. 1962. Discussions in cytogenetics. Burgess Pub. Co., Minneapolis, USA.
10. W. Gottschalk and M. Milutinovic. 1973. Trisomics from desynaptic mutants. The Nucleus., **16**: 1-10.
11. T. Tsuchiya. 1964. Chromosome aberrations and their use in genetics and breeding in barley-trisomics and aneuploids. Barley Genet. 1. (Proc. Irst. Int. Barley Genet. Symp.) : 116-150.
12. T. Tsuchiya. 1967. Establishment of a trisomic series in a two rowed cultivated variety of barley. Can. J. Genet. Cytol., **9**: 667-682.
13. R. G. Palmer and H. Heer. 1976. Aneuploids from desynaptic mutant in soybean (*Glycine max* (L.) Merr.) Cytologia., **41**: 417-427.
14. E. J. Gardner and D. P. Snustad. 1984. Principles of Genetics. John Wiley and Sons, Newyark: pp 475.
15. R. T. Ramage. 1985. Cytogenetics. *In*: Barley (ed. Rasmusson), Amer Soc. of Agron, Crop Sci. Soc. of Amer., Soil Sci. Soc. of Amer., Publishers. Madison, Wisconsin, USA, pp. 127-154.
16. K. Das and G. prasad. 1982. Cytogenetical studies on some barley interchanges and trisomics. *In*: Advances in Cytogenetics and Crop Improvement. (eds.) R.B. Singh, R.M. Singh and B.D. Singh, Kalyani Publishers, New Delhi, India, pp. 145-149.
17. T. Tsuchiya. 1954. Trisomics in barley. Jap. J. Genet., **29**: 179.
18. T. Tschiya. 1961. Cytological identification of the extra chromosomes in crosses with Burnham translocation testers. Jap. J. Genet., **36**: 444-451.
19. K. Das and H.M. Srivastava. 1969. Interchange trisomic in barley. Genetica., **40**: 555-565.
20. K. Das and G. Bhowmik. 1971. A primary trisomic for chromosome 4 in six rowed barley. Cytologia., **36**: 674-679.
21. K. Das and S. C. Kundu. 1973. A primary trisomic for chromosome 3 in six rowed barley. Cytologia., **38**: 91-97.
22. G. Prasad and K. Das. 1976. Interchange trisomics in six rowed barley. Cytologia., **40**: 627-632.
23. T. Tsuchiya. 1963. Trisomics in a cultivated two rowed barley variety. Barley Newsl., **6**: 99-100.
24. G. Ostergren and E. Vigfusson. 1953. On position correlation of univalents and quasi-bivalents formed by sticky univalents. Hereditas., **39**: 33-50.
25. H. M. Srivastava. 1974. Radiation induced desynaptic mutant in barley. Cytologia., **39**: 63-68.
26. J. O. Beasley and M. S. Brown. 1942. Asynaptic *Gossypium* plants and their polyploids.J. Agr. Res., **65**: 421-427.
27. R. P. Celarier. 1955. Desynapsis in *Tradescantia*. Cytologia., **20**: 69-83.