ARTIFICIAL INDUCTION AND PRACTICAL VALUE OF TETRAPLOID SOYBEANS

I. Morphological and Cytological Observations[1]

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Soybean autotetraploids was first successfully obtained by artificial induction and their morphological and compositional characteristics briefly described by P. S. Tang and S. W. Loo (1940). Since then, Porter and Weiss (1948) and Sen and Vidyabhusan (1961) reported more detailed observations and presented discussions on some related problems. These merely marked the opening of a new field for soybean breeders. Much more is to be learned regarding a simpler and more efficient technique of induction, the rating of variations in variaties at tetraploid level, the cytological analysis and economic evaluation of polyploid progenies, and studies on phylogenetic affinities of induced polyploids to wild tetraploid soybeans such as *Glycine tomentosa* and *G. tomentella* securred in nature.

Material and Methods

Colchicine-lanolin mixture at 4 different concentrations, 0.1, 0.2, 0.3 and 0.4% was used in the test as an inducing medium. Young seedlings with unfolded primary leaves, of 4 soybean varieties (Mamottan, CNS, Japanese Green and San Kau) were tested. After treated at apical buds with the inducing medium by brushing, the seedlings were transferred from greenhouse to a cool shaded place for 2 days so as to minimize any harmful effect by the chemical. The treatment took place in July 1961. All plants were grown in pots which were finally put under ordinary cultivation environment. Seeds yielded by the treated plants (C_0 generation) were harvested in mid-November of the same year. For propagation and observations, they were sown on March 30, 1962, partly in pots again and partly in the field.

Prior to the afore-mentioned, a preliminary test by similar technique was conducted in 1960 and a few tetraploid seeds of the varieties Mamottan and CNS were obtained therefrom. These seeds took part in the 1961 test too.

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For cytological observations, both flower buds and root tips were used. The former was fixed in freshly prepared Farmer's fluid for 16–20 minutes and thence preserved in 70% alcohol under 0°–5°C. temperature. For PMC studies, propionic carmine was used. The root tips were pre-treated with a saturated solution of para-dichlorobenzene for 2–4 hours at 15–20°C. gently warmed in 1N. solution of HCl for 5–10 minutes and then squashed with aceto-carmine.

Experimental Results

(*1*) *Percentage of Successful Induction:*

Of the 4 soybean varieties tested, the average percentage of successful induction varied from 25 (San Kau), 27.5 (Mamottan), 32.5 (Japanese Green) to 37.5 (CNS). Hence CNS appeared to be the most sensitive variety in response to artificial induction, but its C_0 generation produced very few pods and seeds. On the other hand, the variety San Kau was the least sensitive but produced much more pods.

The effectiveness of the 4 different concentrations of the inducing medium varied also. The 0.3% concentration gave the best result (successful treatment 46.6±5.2%). Probably the 0.1 and 0.2% concentrations (successful treatment 26.0±1.9% and 36.6±9.8% respectively) were not strong enough to effectively induce the chromosome doubling, whereas the over-dosage by the 0.4% concentration (successful treatment 26.0±1.9%) led to serious injuries to the apical and even lateral buds.

(*2*) *Seed Germinability*

The germination of tetraploid seeds, both in percentage and in germinating rate, appeared to be practically the same as that of the diploid seeds. It was also found that fully matured tetraploid seeds, when properly dried and stored, would not suffer any appreciable loss in germinability within one year. These findings are not in accord with that found by Sen *et al.* (1960) who noted the significantly low germinability in tetraploids and attributed it to the slower rate of moisture absorption.

(*3*) *Morphology of the Stem:*

Since colchicine has certain distorting effects on young seedling, the earlier developmental stages in the C_0 generation were depressed and abnormal. Morphological comparisons against the diploid counterparts were therefore based upon C_1 generation.

In general, the stem of tetraploids with determinate growth habit, had more profuse pubescence and slower growth rate than that in diploids. The tissues were coarser and the nodes at times swelled up like a gall. The branches were, with the exception of the variety San Kau, much fewer in number than

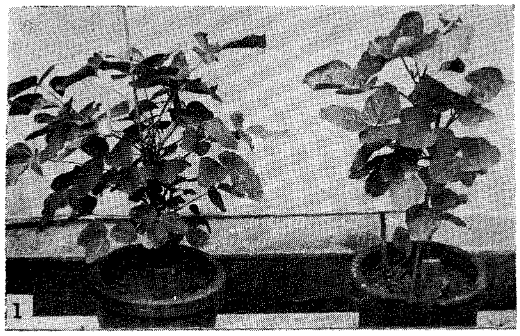


Fig. 1. General features of the diploid (left) and tetraploid (right) plants of CNS.

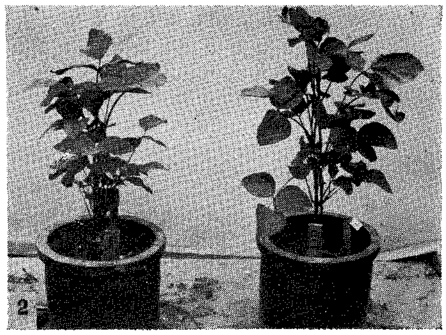


Fig. 2. General features of the diploid (left) and tetraploid (right) plants of Mamottan.

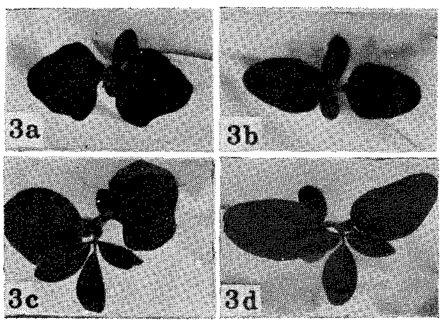


Fig. 3. Primary leaves.
3a. Tetraploid of Mamottan.
3b. Diploid of Mamottan.
3c. Tetraploid of Japanese Green.
3d. Diploid of Japanese Green.

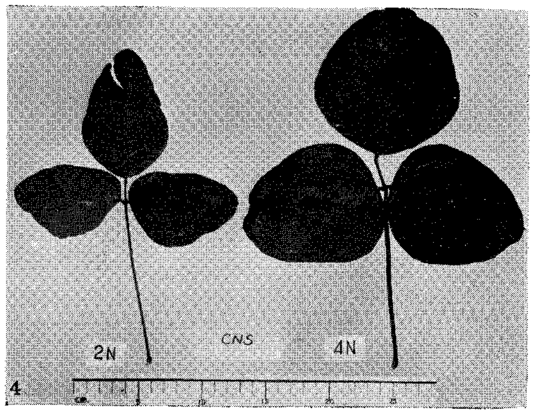


Fig. 4. Compound leaves of diploid and tetraploid plants of CNS showing differences in the size and shape of the leaflets and in the length and thickness of the petiole.

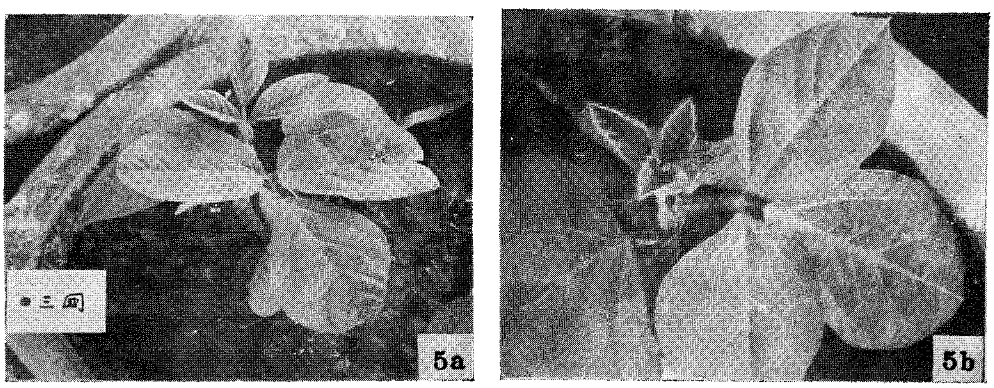


Fig. 5. First compound leaf of diploid (5a) and tetraploid (5b) plant of the variety San Kau, showing the difference of their pubescence on leaflets and petiole.

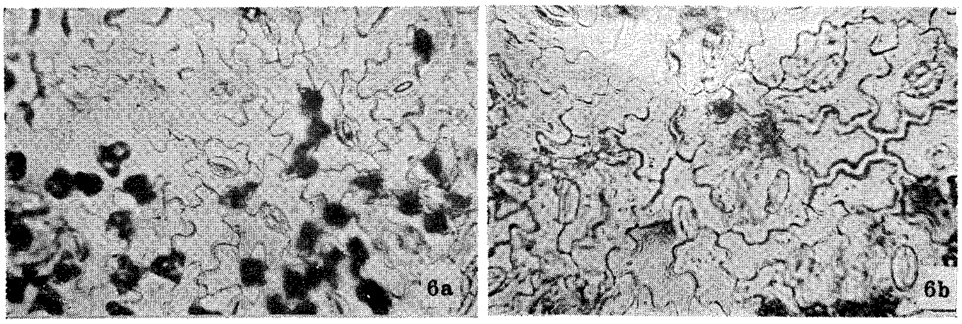
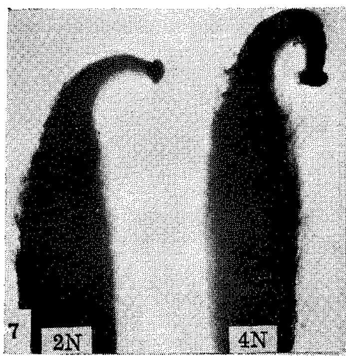


Fig. 6. Leaf stomata of diploid (6a) and tetraploid (6b) plants of variety San Kau.



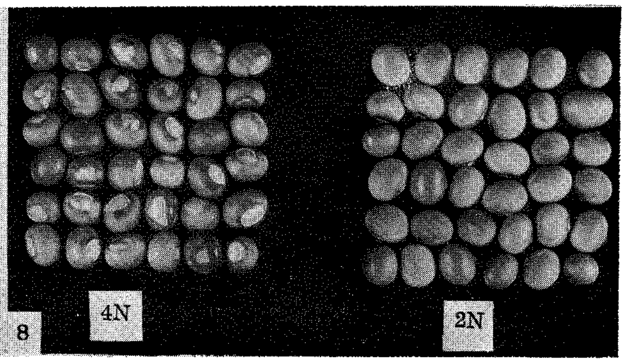
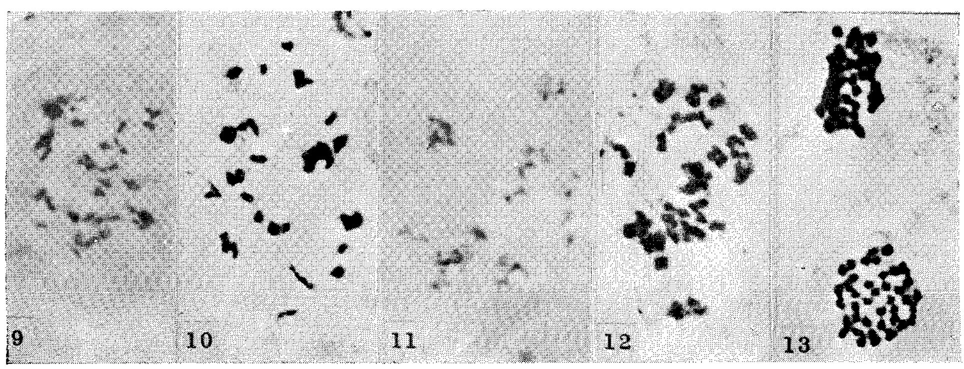


Fig. 7. Styles of the variety Mamottan, showing the apical bending and pubescence.

Fig. 8. Matured seed of the variety Mamottan.



Figs. 9-11. Chromosome behavior of the tetraploid pollen mother cells of the variety CNS at metaphase I. (1000×)

Figs. 12-13. Chromosome behavior of the tetraploid pollen mother cells of the variety Mamottan. (1000×) 12. Metaphase I. 13. Anaphase I, n=40.

those in diploids and many of these branches were sterile. The branching date was about 15–20 days later than that in diploids and the plants often died off before all their pods became matured.

The height of tetraploid plants varied with the variety in question. In San Kau, they were taller than their diploid counterpart throughout the growing period; in Mamottan, taller at first but slightly shorter at later developmental stages; and in the 2 remaining varieties, shorter at all time.

(4) *Morphology of the Leaf:*

The cotyledons of tetraploid soybeans were heavier in texture and in

Table 1. *Behavior and physical characters of the 4 soybean varieties tested, showing similarities and dissimilarities in di- and tetra-ploid plants.*

Characters \ Varieties		CNS	Japanica Green	Mamottan	San Kau
Germinability (%)	2N	94.5	81.2	91.4	92.9
	4N	60.9	80.5	90.2	92.5
Days required, unfolding of primary leaves[1]	2N	5.0±0.13	5.0±0.12	7.0±0.12	5.0±0.13
	4N	7.4±0.09	6.8±0.19	7.0±0.10	7.4±0.09
Total No. of branches per plant	2N	6.4±1.36	3.4±0.24	4.0±0.32	1.4±0.45
	4N	3.6±0.39	3.2±0.30	2.5±0.24	9.2±0.41
No. of sterile branches per plant	2N	2.6±0.40	0	0.2±0.20	—
	4N	3.2±0.45	1.2±0.36	1.7±0.27	—
Height of plant, 40 days after sowing (cm)	2N	18.4±0.15	21.5±0.25	12.1±0.17	9.5±0.14
	4N	15.3±0.19	21.1±0.51	19.5±0.15	12.6±0.76
Height of plant, 90 days after sowing (cm)	2N	27.9±0.24	36.4±0.41	24.7±0.35	54.2±0.74
	4N	25.5±0.32	32.2±0.37	21.2±0.17	54.7±1.37
Length of apical leaflet (cm)[2]	2N	11.9±0.57	9.7±0.03	14.5±0.10	10.7±0.43
	4N	12.8±0.29	10.0±0.13	15.5±1.23	11.2±0.24
Width of apical leaflet (cm)[2]	2N	7.2±0.37	6.1±0.08	8.5±0.06	6.7±0.21
	4N	11.8±0.19	8.8±0.15	14.4±0.13	10.2±0.13
Length/width of apical leaflet (cm)[2]	2N	1.6±0.04	1.5±0.03	1.7±0.05	1.5±0.05
	4N	1.0±0.03	1.1±0.03	1.1±0.04	1.0±0.04
Date of initial blosoom, days after sowing	2N	48.0±0.20	46.0±0.20	41.4±0.11	61.6±0.10
	4N	50.1±0.34	49.8±0.47	41.5±0.11	61.6±1.08
Total No. of pods per plant	2N	130.4±2.01	60.8±1.53	35.0±1.25	—
	4N	31.9±1.98	27.1±1.21	5.9±0.78	—
No. of pods (length 2 cm or more) per plant, 67 days after sowing	2N	37.3±0.42	53.8±0.84	37.8±1.45	—
	4N	6.6±0.72	11.0±0.94	10.7±1.35	—
No. of 1-seeded pods per plant	2N	21.4±2.51	4.6±0.95	4.8±0.53	—
	4N	8.3±1.79	4.8±0.89	4.4±0.3	—
No. of 2-seeded pods per plant	2N	83.0±2.38	29.6±1.52	18.61±.58	—
	4N	17.4±1.73	12.9±1.98	5.2±0.75	—
No. of 3-seeded pods per plant	2N	26.0±1.91	26.6±1.10	11.7±1.96	—
	4N	6.18±2.31	6.46±1.26	0.9±0.89	—
No. of matured seed per plant	2N	22.0±3.56	123.8±1.33	55.4±3.56	—
	4N	7.1±4.03	52.2±5.84	7.4±4.35	—
Weight of 100 matured seed (gr)	2N	4.4±1.32	14.7±1.02	13.1±1.25	—
	4N	4.9±2.59	7.0±3.01	5.2±2.93	—

1) The days required are counted from the day of sowing.
2) Of fully grown compound leaf.

pigment than that of diploids, and were sometimes twisted inward. In general, the primary leaves spread slower, about 2 days later than those of the diploids. They were larger, thicker and roundish or about as long as wide, not so small, thin and much longer than wide as in diploids. The leaflets were also larger and heavier in tetraploids. While the number of leaflets per compound leaf was greater (one compound leaf often had 3, sometimes even 5 leaflets), the total number of leaves per plant was smaller in tetraploids. In short, because of fewer branches, leaves and mature pods and because of the determinate growth habit in most of the varieties tested, the tetraploids were seemingly stouter but less prolifically foliated.

The guard cells of leaf stomata in the tetraploids were comparativly larger in size but fewer in number. For instance, in the variety San Kau, such cells of the tetra- and diploids were $36.4 \pm 0.9 \times 6.1 \pm 0.5\,\mu$ and $22.1 \pm 0.9 \times 4.8 \pm 1.2\,\mu$ respectively, and their numbers were about in the ratio 7:10.

(5) *Morphology of the Flower:*

The floral organs of tetraploids were larger, more intensely pigmented and much fewer in flower number than those of diploids but the flowering date and flowering period were generally similar to that of the latter. Under field conditions, tetra- and diploid soybeans of the same variety might bloom simultaneously.

The pollens of tetraploids were comparatively larger in size but with higher sterility. For example, in the variety San Kau, 35% of the pollens were sterile (in case of diploids, 5%); and in Japanese Green, 37% (6%).

The stamen in tetraploid soybeans was adorned with long dense pubescence which might partly cover the style. In turn, the style was long and very strongly bent toward its base, probably resulted from over-development as well as its restriction by the closed keel. These unusual characters apparently led to the lower percentage of successful pollination in the tetraploid soybeans.

(6) *Morphology of the Pod and Seed:*

As in stems and flowers, the tetraploid pods were profusely pubescent and intensely pigmented, whereas the seeds were about as large as but more spherical in shape than those of the diploids. The seed coats of many of the seeds, when fully matured, were partly ruptured (Fig. 8). But this did not effect their germinability.

The yield of tetraploid soybeans was significantly low. This was largely due to the very slow development of the floral and seed-bearing organs which was responsible to the high percentage of empty, immature or single-seeded pods. The failure to yield any mature seeds by both tetra- and diploids of the fall variety San Kau might be attributed to the unsuitable sowing season.

(7) *Cytological Observation:*

The chromosomes in pollen mother cells of tetraploid soybeans paired in the metaphase I. The quadrivalents were chain-shaped, and were more numerous than bivalents. Uni- and trivalents could not be found in the material. In the anaphase I, 40 dot-shaped chromosomes clearly definable, but there were no lagging chromosomes or other abnormalities.

Rod-shaped chromosomes of varying lengths (2μ in average) were formed in the metaphase I of the tetraploid root-tip cells. As expected, their number was 2n=40 in the diploid and 2n=80 in the tetreploid plants. Good figures were not obtained for this stage as our material was limited and had been overstained.

Discussions and Summary

1. As an inducing medium for tetraploid soybeans, the 0.3% colchicine-lanolin mixture seemed to be superior to the 0.1, 0.2 and 0.4% concentrations. It successfully effected 46.6±5.2% of the plants treated.

2. In contradiction to the conclusion reached by Sen *et al.* (1960), there appeared to have no appreciable difference in the speed and percentage of the germination in the tetraploid seeds as compared with their diploid counterparts.

3. The giganticism in various organs of the tetraploid soybeans was generalized and interpreted by Sen *et al.* (1960), as a natural sequence of the fact that the threshold of soybeans was at a level higher than diploid, *i.e.*, the cultivated soybeans was a monobasic plant and responded to tetraploidy with characteristic enlarged growth features. The same workers also believed this phenomenon to be comparable to that in *Nicotiana* as pointed out by Clausen (1948). From our test, the giganticism seemed to be variable with individual growth feature and individual soybean variety rather than with a generalized tendancy for all varieties.

4. As far as the C_0 and C_1 generations are concerned, the growth rate of the floral and seed-bearing organs was so slow and the yield was so poor so that the economic value of the tetraploid soybeans can hardly be appreciated.

5. Some sterile and other abnormal plants were noted in the C_0 and C_1 generations, but the occurrence of any true aneuploids require further verification.

6. The fall variety San Kau is to be tested more intensively to see if its inconsistency in exhibiting much vigor but having very poor yield in the C_1 generation was due to the unsuitable sowing season.

大豆多元體之誘致及其利用價值之研究

I 形態學及細胞學觀察

湯文通 林齊強

本研究係利用羊毛脂與秋水仙鹼伴合爲濃度 0.1, 0.2, 0.3 及 0.4%的藥劑，塗抹於大豆幼苗之生長點上，以誘致其發生四元體，共有四個品種（三國、日本青皮、CNS. Mamottan）參加試驗，其結果如下：

（1） 以 0.3% 濃度效力最大；CNS 發生四元植株較多，三國及 Mamottan 較少。

（2） 四元體種子與二元親種種子之發芽力並無差異。

（3） 四元體：莖較粗；分枝較少；葉較厚，圓形；花較大，柱頭彎曲，多毛；種皮破裂。

（4） 四元體之花粉母細胞在分裂中期，可見到成對排列及鏈狀之四價體及二價體。四價體之出現次數多於二價體。至於三價體及單價體未曾明確察出。在分裂下期，可看見很清楚之粒狀染色體，數目 n=40，未曾發現滯留染色體等不正常現象。

（5） 由於四元體植株開花甚不一致，形成多量空莢，又四元體之一粒莢比例高，種子並不較二元體者大，故產量低，無經濟栽培之價值。

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