Somatic Mutation of Tea Plants Induced by γ-Irradiation

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As tea plants are highly heterozygous in genetic composition, great variations occur in plant charatceristics such as leaf shape and growth pattern when propagated by seeds. Therefore, vegetative propagation such as cutting or layering is applied to establish tea fields in recent years. If useful mutants are induced by irradiation, they can be utilized directly for commercial production.

Study on radiation breeding of tea plants was initiated in 1962. The work has been carried out mainly in close cooperation between the Institute of Radiation Breeding and the National Research Institute of Tea on radio-sensitivity, conditions for inducing bud mutation and characteristics of induced mutants, etc.. Results[1)-7)] of the study will be described briefly in this paper.

Radio-sensitivity of tea plants under chronic γ-irradiation

As a basic study for induction of bud mutation, radio-sensitivity of plants grown in the γ-field[8)] of the Institute of Radiation Breeding was examined for a period of 3 years[3),7)]. Two to three years old plants of 13 varieties were planted at varying distances, 11.5–98.5 m, from the radiation source (^{60}Co) with an interval of 1.5 m in April 1962.

Growth inhibition such as reduced number of leaf buds or flower buds was reported with tea plants grown in the γ-field[9)]. Effect of chronic γ-irradiation on plant height is shown in Fig. 1. Plant height was reduced with an increase of exposure rate, giving a correlation coefficient of −0.975**. Irradiated plants produced abnormal leaves, such as elongated leaf, bifurcated leaf or asymmetric leaf, with higher frequency at nearer distance from the γ-source. The frequency was also higher in the second year than in the first year after irradiation.

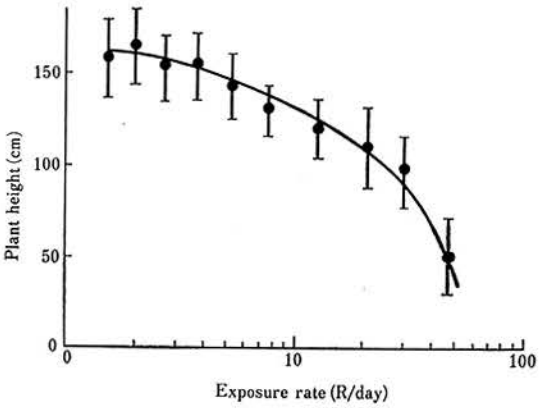


Fig. 1. Plant height of tea plants under chronic γ-irradiation
(result obtained after 2 years and 7 months from planting)

Lethal exposure rate for young plants determined in December 1962–1964 is shown in Table 1. In 1962, a few plants were killed even under relatively high exposure rate, but the lethal exposure rate lowered from year to year. From the 1964 result, lethal exposure and its rate were estimated to be 60–80 KR and 60–80 R/day respectively. However, black tea varieties appear to be less resistant.

Rooting ability of cuttings taken from new shoots of plants grown under chronic irradiation was examined in 1963, by taking cuttings from each planting row of the γ-field and determining their survival in a greenhouse after one year. Cuttings of plants grown at

Table 1. Lethal exposure rates of various tea varieties under chronic γ-irradiation in the γ-field

Variety		Lethal exposure rate (R/day)		
		1962	1963	1964
For green tea	Yabukita	250	130	80
	Tamamidori	250	130	70
	Yamatomidori	>260	60	60
	Yutakamidori	>260	—	60
	Z 1	250	130	80
	Horyoku	140	100	60
For black tea	Benifuji	140	40	30
	Benihomare	250	90	40

Table 2. Number of leaf initials in the winter bud and expanded leaves in the first flush period of tea plant irradiated chronically

Exposure rate	No. of leaf initials in the winter bud		No. of expanded leaves in the spring of 1971	Total exposure (from Nov. to March)
	Counted in November, 1970	Counted in March, 1971		
R/day				R
40	3.9	3.6	3.7	6000
22	—	4.4	4.8	3300
10	5.1	5.1	5.5	1500
5	5.1	5.4	5.8	750

the exposure rate more than 40 R/day gave only 50% of survival, whereas that grown with less than 30 R/day gave more than 70%.

Mutation induced by chronic irradiation

With plants growing in the γ-field, bud mutation induced by chronic irradiation and factors affecting the induction were studied[4]. At two years after planting under 6–50 R/day of exposure rate, mutants with different leaf color occurred. As shown in Fig. 2, frequency of mutated shoots with variegation was high at about 20–30 R/day or 6–8 KR/year with mutation rate per plant of 12% at the highest.

Such mutations were localized to a particular portion of plants, i.e., mostly to the second flush region of shoots, but the first flush region was normal. It is usual that shoots on unplucked tea plants exhibit more than two cycles of growth. The second flush grows from the terminal buds on the first flush shoots. However, plants grown under the exposure rate of 20–40 R/day produced so-called blind shoots which were lacking terminal buds, and the second flush grew from upper axillary buds of the blind shoots. Mutation occurred

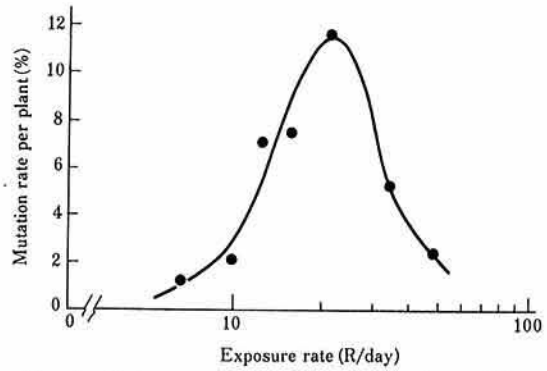


Fig. 2. Frequency of bud mutations observed in tea plants under chronic γ-irradiation, as represented by the average of eight varieties

on such second flush shoots.

Winter buds of tea plants are usually composed of 4–7 leaf initials and the number of leaf initials has close relation to the number of leaves to be developed in the first flush. Under a chronic irradiation, the higher the exposure rate the less was the leaf initials as well as leaves expanded in the first flush (Table 2), indicating the radiation damage of apical meristem of winter buds, which causes missing terminal buds on the first flush shoots. The damage of winter buds was confirmed by histological observations, as reported by Futsuhara[10]. In general, it is desirable to induce mutations at the meristem composed of the least number of initial cells in order to get whole mutants. Histological examination of irradiated winter buds indicated that the differentiation of new buds which produce the second flush shoots was hardly going in the axillary region adjacent to the damaged apical meristem. Presumably mutations occurred in such winter buds under continuous irradiation.

Effect of heavy pruning of induction of mutations by γ-irradiation

With various kinds of plants, a number of whole mutants were obtained by developing new shoots from latent buds by repeatedly applying heavy cut back after γ-irradiation. Effect of such cut back treatment was examined with tea plants[6].

Tea plants growing in pots were subjected to the γ-ray of 2.5 KR or 5 KR for a period of 6 days, before or after the heavy pruning applied at 10 cm above the ground surface. As a result, mutation occurred at a rate of 20% per plant and 4% per shoot. Heavy pruning applied before the irradiation gave slightly higher rate. Heavy pruning applied in July gave less shoots developed and less frequency of mutation than that applied in March. This may be due to the difference in carbohydrate reserve required for the recovery after pruning. Mutations obtained were elongated leaf, small leaf, large leaf, yellow leaf, variegated leaf and their combinations (Plate 1).

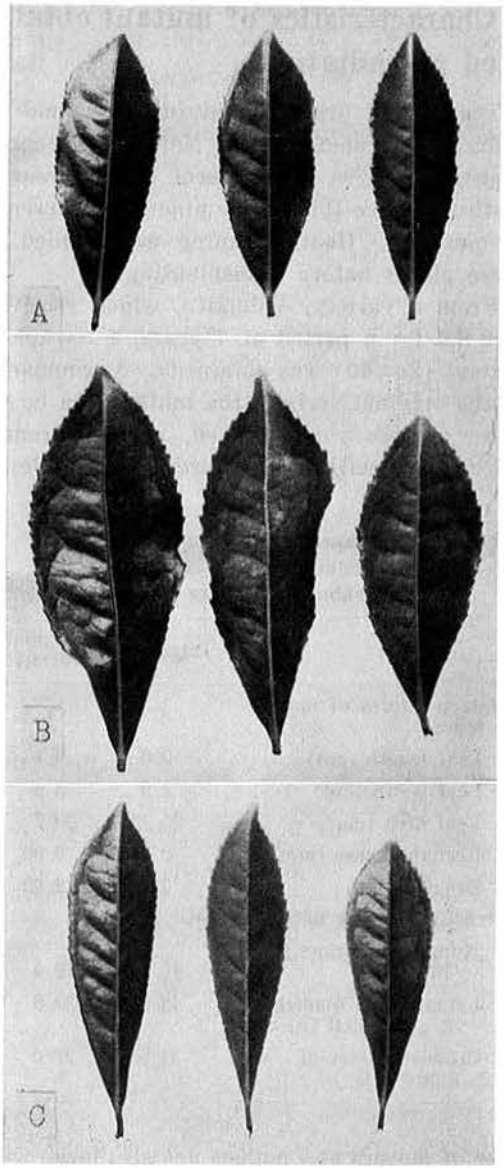


Plate 1. Kinds of the somatic mutations of the plant induced by the pruning treatment
A: original variety (Z 1)
B: large leaf
C: elongated leaf

From this result, it is considered that heavy pruning in March followed by γ-irradiation may be most effective in inducing bud mutation of tea plants.

Characteristics of mutant obtained γ-irradiation

Tea plants, first planted in the γ-field in 1962, were transferred to National Research Institute of Tea and several other research institutes since 1965 to examine the occurrence of mutation. Heavy pruning was applied to these plants before transplanting.

From a variety, Yabukita, which received 17.9 KR for a period of 7 years, a tetraploid mutant (2n=60) was obtained[1]. As compared to the original veriety, the mutant has large, more or less round-shaped, and apparently thick leaves, with large guard cells. (Table 3)

Table 3. Characteristics of tetraploid tea induced from γ-irradiated Yabukita variety

	Tetraploid	Original variety
External form of mature leaves		
Leaf length (cm)	9.0	9.1
Leaf width (cm)	3.9	3.2
Leaf area (cm^2)	25.1**	20.7
Leaf thickness (mm)	0.45	0.36
Length/width	2.29**	2.80
Density and size of stomata		
Number of stomata (10×10)	10.1**	19.4
Longitudinal diameter of guard cell (μ)	43.9**	33.8
Cross diameter of guard cell (μ)	34.5**	27.0

Size of flowers and pollens are also large with 50% fertility. Plucked shoots contain less tannin and more total nitrogen and aminoacids than that of original variety.

One of the mutant strains obtained by γ-irradiation followed by heavy pruning of a variety, Yutakamidori, showed a change in aminoacid composition (Fig. 4). Among aminoacids contained in normal tea shoots, content of theanine is highest, followed by asparatic acid, glutamic acid, and arginine. In the mutant strain, content of arginine was found to be as high as two times that of the original variety. Since arginine has a close relation to the taste of tea, the occurrence of such a mutant by γ-irradiation is noteworthy in the breeding of tea for better quality.

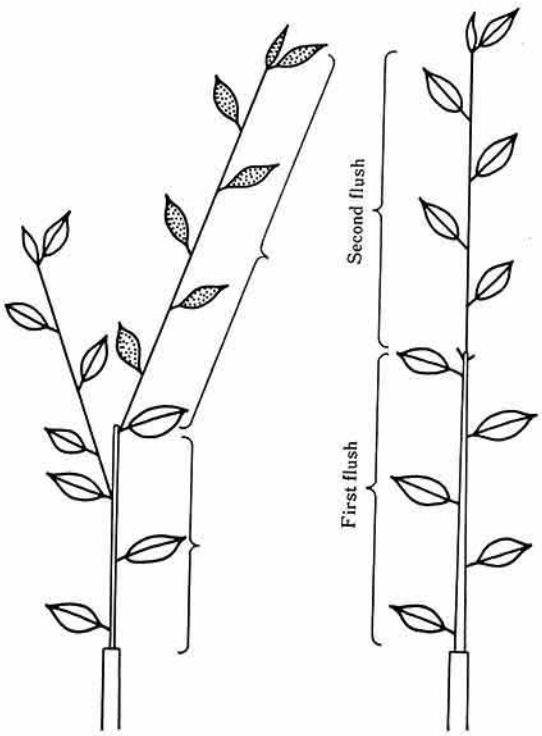
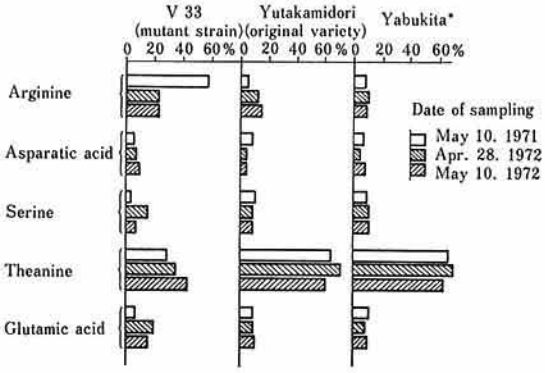


Fig. 3. A schematic representation of the mutated portion of the tea shoot grown under chronic irradiation in the γ-field



* The leading variety for green tea in Japan

Fig. 4. Amino acid content in the new shoot of a mutant tea strain (ratio to total)

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