IMPROVEMENT OF GRASSES THROUGH INDUCED CHROMOSOMAL RECOMBINATIONS

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ABSTRACT

IMPROVEMENT OF GRASSES THROUGH INDUCED CHROMOSOMAL RECOMBINATIONS

by Don J. Heinz

Polyploid and structural hybrid systems have evolved naturally giving certain genera greater survival value over that of ancestral types. Consideration should be given to the use of these systems in applied breeding. The purpose of this study was to determine 1) the effects of irradiation (source of irradiation was neutrons) induced translocations in tetraploid Dactylis glomerata and 2) the results of induced polyploidy in Lolium species.

Irradiation in Dactylis resulted in a wide range of translocations, as measured by the number of multivalent chromosome associations of more than four at diakinesis. One-half-hour of irradiation was more efficient than the one-fourth or one-hour treatments in the induction and survival of translocations. The morphological and fertility characteristics of the N_2 population (one-half-hour treatment) were not affected. Three plants heterozygous for a large number of translocations and more vigorous than any found in the control population were isolated. There was a significant difference at the 20% level between the three largest plants heterozygous for translocations and the three

largest control plants.

A breeding system for polyploid forage grasses, based on structural hybridity has been proposed.

Seed from many inter- and intraspecific crosses of Lolium multiflorum and L. perenne were treated with colchicine. Also, part of the seed was given an irradiation treatment in addition to the colchicine treatment. Tetraploid sectors were obtained from mature plants derived from treated seed. Progeny obtained from corresponding diploid and tetraploid clones were studied in the field.

Greater rust resistance was found in induced tetraploid Lolium multiflorum populations over corresponding diploid populations. Some tetraploid plants were more vigorous than any found in the diploid populations.

The vigor of Lolium multiflorum was combined with the rust resistance of L. perenne in diploid and tetraploid hybrids. Tetraploid hybrids in the first generation were not superior to corresponding diploid hybrid populations. There were more differences between the original clonal lines than between the corresponding diploid and tetraploid populations. However, further crossing and selection might result in progress at the tetraploid level.

Close agreement was found between autotetraploid Lolium multiflorum and the hybrids of L. multiflorum X L. perenne in various meiotic configurations, suggesting that cytogenetically the two species do not have many chromosomal differences.

IMPROVEMENT OF GRASSES THROUGH INDUCED CHROMOSOMAL RECOMBINATIONS

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INTRODUCTION

There are many grass species in use on farms, ranches, and rangelands in the United States which have been derived directly from introductions or native species. Some species have been improved through breeding, but progress has been slow. Detailed genetic and cytogenetic information is limited and only a few have been studied to any great extent. Also, there is a general lack of agreement concerning the evaluation and definition of desirable forage characteristics.

Some gains have been made through breeding in disease resistance, leafiness, seed yields, and date of maturity, while total dry weight yields remain constant. Most new varieties being released are not significantly higher in yield from those already in use.

A better knowledge of the genetic system of the grasses is needed. This associated with new methods and techniques should give greater progress through breeding.

Polyploid and structural hybrid systems have evolved naturally giving certain genera greater survival value over that of ancestral types. Consideration should be given to the use of these systems in applied breeding. Efforts to apply these techniques through artifical means have failed in many cases. Lack of success was most likely due to short range programs, with immediate improvement the goal. When

immediate success was not obtained, the technique was written off as not being of value. New techniques should be used in combination with other methods to give superior plant types.

The purpose of this study was to determine 1) the effects of radiation-induced translocations in tetraploid Dactylis glomerata, and 2) the results of induced polyploidy in Lolium species.

REVIEW OF LITERATURE

Cytogenetics and Breeding of Forage Grasses. Several reviews have been published on the subjects of genetics, cytogenetics, and breeding problems in perennial forage grasses. The divergence of opinion held by forage breeders concerning the objectives to be attained and breeding methods used in the improvement of forage grasses have been reviewed by Hanson and Carnahan (1956).

Cytogenetic and genetic studies made on forage grasses have been reviewed by Myers (1947) and Carnahan and Hill (1961). Myers lists two reasons for the importance of cytogenetical studies in forage grasses: "(a) to serve as an adjunct to morphological data in studies of taxonomy and phylogeny and (b) to provide fundamental information for the improvement of species by breeding."

Effects of Irradiation on Plant Yields. A large number of studies have been made concerning the effects of ionizing radiations on plants and their use in plant breeding (Mac Key, 1956; Konzak, 1957; Sax, 1957; Sparrow, 1957; Elliott, 1958; Gaul, 1958; and Smith, 1958).

Increased yields through selection within irradiated populations have been reported by several workers. Gregory (1955, 1956) was able to demonstrate a measurable gain in

peanut yields as a result of selection within x-irradiated populations for more productive types. He attributed this gain in yield to the induction of valuable mutations.

Humphrey (1954), studying the effects of thermal neutrons on soybeans, noted a number of lines with increased vigor, greater shattering resistance or both, and one line with significantly higher yields. Papa et al. (1961) reported no substantial yield increase from selection within irradiated soybean lines, but did find several high yielding progenies from irradiated plants that combined high yield with additional desirable qualities.

Mac Key (1954) reported improved agronomic characteristics in wheat as a result of radiation induced deficiency duplications. He also reported increased yields in wheat and oat lines selected from irradiated populations.

Gelin (1954) released a new higher yielding pea variety selected from irradiated lines. He associated the increased yields with induced mutations.

Andersson and Olsson (1954) reported the release of a higher yielding white mustard variety as a result of selection within irradiated lines.

Evolution of Structural Hybridity. Structural hybridity in the form of translocations has played an important evolutionary role in certain plant genera. The most studied of these groups has been Oenothera, in which structural hybridity is a dominant and important feature. Stebbins (1950)

and Burnham (1956) list a number of other plant genera possessing similar structural features, but not as extensive or complex as found in Oenothera.

Swanson (1957) has presented the main features of the Oenothera system in a short and concise manner. Basically the system evolves around the formation of a ring of 14 chromosomes at meiosis due to the accumulation of translocations. Alternate disjunction occurs giving two complexes (called gaudens and valens in O. lamarckiana and regins and curvans in O. muricata) of seven chromosomes which function as separate linkage groups. The translocation heterozygote has been maintained through the evolution of a balanced lethal system and self fertilization. In Oenothera lamarckiana the balanced system acts as a zygotic lethal, as contrasted to a gametic lethal system in O. muricata. Only translocation heterozygotes are formed.

Cleland (1950) has summarized the evolutionary features of Oenothera. On the basis of extensive cytogenetic studies he has proposed the following steps in the evolution of structural hybridity in Oenothera.

(1). The appearance and incorporation of translocations until all 14 chromosomes were in a single ring at meiosis.

(2). The appearance of lethals, both gametophytic and zygotic. When these were coupled with large rings the population tended to increase in the number of heterozygous individuals, until the presence of only heterozygotes was

assured through balanced lethals.

(3). The establishment of self fertilization. This may have helped overcome sterility, which probably was associated with balanced lethals and large rings of chromosomes.

(4). The reduction in flower size, making the plant less efficient for insect pollination.

The occurrence of large rings, balanced lethals, and self fertilization in Oenothera gives a high survival rate, but severely restricts chromosome or genetic recombination.

Several cases of the artificial formation of structural hybrids have been reported as in Campanula persicifolia (Darlington and Gairdner, 1937) and corn (Burnham, 1956), but only a portion of the total chromosome complement was involved in large rings.

Nishimura and Kurakami (1953) reported the production of large rings in barley by successive x-ray treatments in an effort to apply the Oenothera system to barley.

Yamashita (1951) produced a structural heterozygote in einkorn wheat involving all 14 chromosomes in one large ring.

Cytogenetic Studies in Dactylis. A number of cytogenetic studies have been made on Dactylis. Quadrivalent formation at diakinesis and metaphase I have been reported by a number of workers. Müntzing (1933 and 1937) reported an average of 3.48 and 3.5 quadrivalents per microsporocyte. He reported no pollen mother cells with greater than quadri-

valent associations.

Myers and Hill (1940) reported an average of 3.3, 3.8, and 4.2 quadrivalents per microsporocyte for three plants. From a study of twenty plants, Myers and Hill (1942) reported the average number of quadrivalents per sporocyte ranged from 2.42 to 4.39. Myers (1943) found a range of 3.0 to 4.09 quadrivalents in nine plants.

McCollum (1958) reported a mean number of 3.44 quadrivalents at metaphase versus 3.5 at diakinesis for artificially induced tetraploids from crosses between diploid subspecies of Dactylis.

Müntzing (1937) reported uneven distribution of chromosomes in 15.3% of the microsporocytes examined at anaphase I. Myers (1943) observed unequal distribution of chromosomes in 13 out of 520 microsporocytes examined at anaphase I.

Myers and Hill (1942) found that the number of microsporocytes with univalents varied from 3.4 to 13.8% per plant at metaphase I, and from 1.7 to 34.0% of microsporocytes of different plants at anaphase I had lagging chromosomes. Myers (1943) found no correlation between the number of quadrivalents found and number of univalents at anaphase, but reported a correlation between univalents at metaphase I and laggards at anaphase I.

The number of bivalents may range from 0 to 14 and the frequency of quadrivalents from 7 to 0 per microsporocyte. There have been no reports of multivalents found with greater

than four chromosomes.

Irradiation Studies in Dactylis. There are only a few reports on the effect of irradiation in orchardgrass. Stebbins and McCollum (1955) reported the induction of translocations by x-irradiation on diploid Dactylis. Zijp (1960) reported the possible induction of a mutant giving a plant free of silica deposits on the leaves.

Mac Key (1956) mentions that mutation studies are being conducted on orchardgrass by workers in Sweden. Osborne (1957) lists several workers who have had orchardgrass seed irradiated with neutrons, but no reports have been published on their work to date.

Polyploidy Studies in Lolium. Polyploidy has been an important factor in the formation of new races and species in nature. Many of our most important crop plants are of polyploid origin. Carnahan and Hill (1961) estimated that of some 2,300 grass species for which the chromosome numbers are known, approximately 80% are polyploid. Lolium is one of few genera in the Gramineae in which all known species are diploid (2n=14). In recent years interest has been shown in polyploidization of Lolium species for the improvement of certain agronomic characteristics.

Mehta and Swaminathan (1957) have reviewed studies on the induction of polyploidy in forage crops, including work with Lolium. Therefore, this review will be limited to recent work in polyploidization of ryegrass.

Wit (1958) and Hertzsch (1959) reported the production of large numbers of tetraploid Italian ryegrass plants. They found higher green weights, a lower total percentage of dry weight, lower numbers of leaves and of culms in the tetraploids when compared to their diploid counterparts. They reported a potential for greater winter hardiness in selected tetraploid plants over that observed in diploids. A reduction in fertility was observed in most newly established tetraploid lines.

De Roo (1959) reported that progress could be made for fertility and other important agronomic characteristics by selection in tetraploid lines of Italian ryegrass.

Wit and Speckmann (1954) found greater rust resistance in tetraploid Italian ryegrass after one generation of selection, while in perennial ryegrass rust resistance was improved over the diploid counterpart only after repeated selection.

Wit (1959) reported that tetraploids of Italian ryegrass surpassed the diploids in winter hardiness, forage yield, and seed production; while in perennial ryegrass, cold resistance, forage yield, and rust resistance could be raised to or above the diploid level in as little as one cycle of selection.

The development of induced tetraploid ryegrass varieties has been enhanced by crossing doubled parents (induced tetraploids) from several sources of germplasm. In fact, Wit (1959) has submitted the first tetraploid Italian rye-

grass varieties for registration in the Netherlands.

The production of hybrid varieties from interspecific crosses of Lolium multiflorum X L. perenne has been of interest in recent years. Through selection and stringent seed certification programs, the two species have developed into distinct morphological types. L. multiflorum is characterized as an annual or short lived species producing a palatable, high yielding growth, while L. perenne is a perennial species, less palatable, and with less herbage.

The two species are completely interfertile, and from natural or controlled crosses superior plants can be obtained combining the desirable qualities of both species. However, upon further cross pollination among the hybrids, segregation occurs giving parental types and inferior intermediate types. Several hybrid varieties have been released at the diploid level resulting from selection within large progenies, in combination with strict standards for seed multiplication.

Corkill (1945) was successful in producing a hybrid variety between the two species combining their desirable characteristics. He mentioned that strict controls must be maintained during seed multiplication, as there was a tendency for early flowering, less desirable types to become predominant in the variety.

Kuchař (1957), Desroches (1953), and the Dutch seed establishment of H. Mommerstug (1957) have reported the successful production and selection of desirable hybrids that may be useful as varieties.

Although several workers have tried to combine the desirable characteristics of Lolium multiflorum and L. perenne, there has been no report of utilizing polyploidy for this purpose.

PART ONE

IRRADIATION STUDIES IN *DACTYLIS*

MATERIALS AND METHODS

Source material for this study was obtained from six superior clones selected from individually spaced plants of orchardgrass from P.I. 237174. Open pollinated seed was harvested from all six plants. Equal numbers of seed were then selected from each plant for control and irradiated groups.

The seed was irradiated at the Oak Ridge, Tennessee, facilities of the Atomic Energy Commission, courtesy of T. S. Osborne of the UT-AEC Agricultural Research Laboratory. A total flux of 7.7×10^8 N/cm^2/sec was administered for one-fourth, one-half, and one-hour treatments. Table 1 gives a breakdown of the neutron source. Of the "effective" flux, 93.9% was between 0.025 Mev (epithermal) and 8.6 Mev (fast). The amount of gamma contamination was unknown.

Neutrons were used as the irradiation source, since it has been shown previously by several workers (Caldecott, 1955; D'Amato *et al.*, 1958; and Larter and Elliott, 1956) that a higher frequency of translocations resulted per irradiated plant surviving than from other sources of ionizing radiation.

TABLE 1. Neutron source for P-11 UT-AEC: West animal tunnel, graphite reactor, ORNL, with U^{235} plate

Monitor Foil	Foil Capture Threshold	Flux: Neutrons/cm^2/sec	Percent of Total Flux	Rep/hr	Percent of Total Rep
Au	$2.5 \overline{\text{ev}} \times 10^{-2}$ (Thermal)	4.2×10^8	54.5	43.2	2.9
Pu	2.5×10^4	1.9×10^8	24.7	20.8	19.0
Np	7.5×10^5	1.1×10^8	14.3	705.6	47.8
U	1.5×10^6	3.5×10^7	4.5	252.0	17.1
S	2.5×10^6	1.3×10^7	1.7	147.6	10.0
Al	8.6×10^6	2.4×10^6	0.3	46.8	3.2
	Totals:	7.7×10^8	(100.0)	1476.9	(100.0)

After Dr. Thomas S. Osborne (Personal Communication)

Control and irradiated seed (herein referred to by the letter N) were germinated under controlled conditions (30° C. for 8 hours and 20° C. for 16 hours, with an eight-hour light period) for 21 days. The seedlings were individually transplanted into two-inch peat pots filled with a 1:1 mixture of peat and sand containing essential growth nutrients. When a majority of the seedlings had approximately 15 to 20 leaves, they were vernalized for three months in a refrigerator maintained at 35 to 40° F., with a nine-hour light period every 24 hours.

The progeny (F_1 and N_1) obtained from plant number two was used to carry out this study. Three hundred seed were designated control, with 120 in each of the one-fourth, one-half, and one-hour N treatments.

The seedlings were transplanted into six-inch pots after vernalization. Early in the spring of 1960 the plants were hand transplanted to the field. Each treatment was placed in an isolated block to insure against contamination from outside sources of pollen.

Two panicles on each F_1 and N_1 plant were tagged during the period of heaviest anthesis. Seed for the F_2 and N_2 populations were harvested from these tagged panicles. These same panicles were also used to determine fertility.

Sixty seed from the following number of F_1 and N_1 plants were germinated and the seedlings were used to make up the F_2 and N_2 populations:

Control: 75 plants

Irradiated ¼ hour: 54 plants

Irradiated ½ hour: 75 plants

Irradiated 1 hour: 54 plants

Germination conditions were the same as for the F_1 and N_1 populations. The seedlings were transplanted into two-inch peat pots and allowed to develop in the greenhouse under a 24 hour light regime prior to mechanical transplanting to the nursery on September 10, 1960, water being applied in the process. Fertilizer was applied at the rate of 350 pounds of 15-15-15 per acre in the spring of 1961.

The field design consisted of three completely randomized blocks. Each block had 258 plots with eight plants per plot from one of each of the F_1 and N_1 parents. The plants were individually space planted on two-foot centers in three-foot rows. Thus, there were 24 progeny represented in the design from each of the F_1 and N_1 plants included in this study.

The percentage of stainable pollen was ascertained in the F_1 and N_1 populations at the time of anthesis. Pollen from one panicle branch was shaken into aceto-carmine, and a cover slip placed over the preparation. An immediate count was made of the stainable and nonstainable pollen from five different positions on the slide.

Percentages of fertility were determined for the F_1, N_1, F_2, and N_2 populations by counting the number of florets and seed set on the three center branches of one pan-

icle per plant.

Survival and vigor ratings were taken in the spring of 1961. All plants failing to survive up to October 1, 1960, were replaced. Vigor was scored on the basis of 0 - 5, with 0 = dead and 5 = most vigorous. For the statistical analysis of spring vigor the ratings were changed to a percentage of vigor.

In the fall of 1961 individual plants were cut approximately 1.5 inches above the crown, weighed, and average plant weights determined.

Statistical analyses of field data from the F_2 and N_2 populations were made on averages for the 24 progeny represented from each original parental clone.

Cytogenetic analysis was made on microsporocytes from florets of individual panicles collected when they were completely out of the boot, between 1:15 and 2:30 P.M. on sunny days. Whole panicles were fixed and stored for up to ten months in Newcomer's solution which consisted of the following:

6 parts 98% isopropyl alcohol

3 parts propionic acid

1 part petroleum ether

1 part acetone

1 part dioxane

Cytogenetic analyses were made from temporary propionic carmine smears of pollen mother cells from plants of the F_1, N_1, F_2, and N_2 populations. Photomicrographs were

made from these preparations.

Duncan's Multiple Range Tests for means with equal (1955) and unequal (1957) replications were used to test differences between means for significance. In these tests, differences required for significance between means vary with the number of means in the comparison. Furthermore, the differences required increase as means further apart in rank are compared for significance. Means in the same range are not significantly different; however, a significant difference exists between means found in different ranges. Critical values for the Duncan's Multiple Range Tests were taken from tables developed by Harter (1960).

All results analysed as percentages were transformed by the arcsin transformations for proportions as worked out by C. I. Bliss and tabled by Snedecor (1956). When the observations in an analysis of variance problem are proportions, homogeneity of variance cannot be assummed because σ_p^2 varies with P (proportion) and with N (number). Homogeneity of variance is necessary for a valid analysis of variance. Both the transformed and actual percentages are presented in the tables.

RESULTS

There were no significant differences in germination between treatments for the progeny grown from the six original plants.

The percentage of germination for the progeny of plant number two which made up the F_1 and N_1 populations was as follows:

Control:	90.7
Irradiated ¼ hour:	85.0
Irradiated ½ hour:	93.3
Irradiated 1 hour:	87.5

The percentage of F_1 and N_1 progeny from plant number two surviving to maturity was as follows:

Control:	81.7
Irradiated ¼ hour:	80.0
Irradiated ½ hour:	85.0
Irradiated 1 hour:	85.8

In general there were very few morphological indications of induced mutations in either generation of the irradiated populations. One plant in one of the N_1 populations showed a chlorophyll deficiency. It was visible early in the spring with a gradual disappearance of the characteristic during the summer. This is what would be expected from cross pollinated plants, since most mutations are recessive and

would not be expressed in the phenotype unless they were homozygous for the new mutant. The only way for homozygosity to occur in these populations would be through selfing or close inbreeding.

The percentage of stainable pollen for the F_1 and N_1 is presented in Table 2. Although significant differences were obtained between populations, the range in percentages of stainable pollen from individual plants of the populations was similar. The control had a range of 65.3 to 98.9%, the one-half-hour treatment 63.8 to 100%, and the one-hour treatment 56.2 to 97.4% stainable pollen. The use of the percentages of stainable pollen as an indicator of induced translocations in individual plants was negated by the similarity found in the range for stainability in the F_1 and N_1 populations.

TABLE 2. Ranked means of the percentage of stainable pollen in the F_1 and N_1

Treatment		Control (F_1)	½ hr. (N_1)	1 hr. (N_1)
Means:	Transformed	74.7	71.5	63.5
	Actual	93.0	89.2	78.8
LSR 1% level[1]				

$\bar{X}$ = 69.89 F value = 24.31** $S\bar{x}$ = 1.18 C.V.% = 10.23

** Significant at the 1 percent level.

[1]All means underlined by the same line are not significantly different from each other. All means not underlined by the same line are significantly different from each other.

The cytogenetic analysis of the F_1 and N_1 population for induced translocations was based on the number of chromosomes associated in multivalents over four. Analysis was made at diakinesis on microsporocytes from 25 plants each of the control, one-half, and one-hour treatments. A total of 2,637 microsporocytes were analysed at diakinesis and anaphase. Tables 3, 4, and 5 give the ranked means for the percentage of chromosomes associated as bivalents, quadrivalents, and multivalents over four per microsporocyte in F_1 and N_1 populations as well as the number of bivalent and quadrivalent associations per pollen mother cell.

TABLE 3. Ranked means of percentage of chromosomes involved in bivalent associations and average bivalent association per microsporocyte in the F_1 and N_1

Treatment	Control (F_1)	1 hr. (N_1)	½ hr. (N_1)
Means: Transformed	53.4	45.4	45.2
Actual	64.2	50.7	51.1
Bivalents/microsporocyte	8.98	7.08	7.06
LSR 1% level			
$\bar{X}$ = 48.0 F value = 20.56**	$S\bar{x}$ = 1.03	C.V.% = 10.77	

** Significant at the 1 percent level.

The number of bivalents and quadrivalents found in microsporocytes from control plants were in agreement with those reported in the literature (see Müntzing, 1933 and 1937; Myers and Hill, 1942; Myers, 1943; and McCollum, 1958).

Figure 1 shows the range in the number of bivalent and quadrivalent associations per microsporocyte observed at diakinesis in the control population. Several plants were observed having microsporocytes with a range of 14 bivalents through seven quadrivalents. Very few univalents or trivalent associations were observed.

TABLE 4. Ranked means of percentage of chromosomes involved in quadrivalent associations and average quadrivalent associations per microsporocyte in the F_1 and N_1

Treatment		½ hr. (N_1)	Control (F_1)	1 hr. (N_1)
Means:	Transformed	39.7	36.1	34.8
	Actual	40.6	35.0	32.9
Quadrivalents/microsporocyte		2.88	2.47	2.28
LSR	1% level	———	———	———
	5% level	———	———	———

$\bar{X}$ = 36.85 F value = 7.69** $S\bar{x}$ = .93 C.V.% = 12.57

** Significant at the 1 percent level.

TABLE 5. Ranked means of percentage of chromosomes involved in multivalent associations over four in the F_1 and N_1

Treatment		1 hr. (N_1)	½ hr. (N_1)	Control (F_1)
Means:	Transformed	22.3	14.3	1.3
	Actual	16.0	7.4	.3
LSR 1% level		———	———	

$\bar{X}$ = 12.63 F value = 54.64** $S\bar{x}$ = 1.44 C.V.% = 56.81

** Significant at the 1 percent level.

Fig. 1. Microsporocytes from the control population showing range in bivalent and quadrivalent formations observed: a. 14 II; b. 12 II, 1 IV; c. 10 II, 2 IV; d. 8 II, 3 IV; e. 6 II, 4 IV; f. 4 II, 5 IV; g. 2 II, 6 IV; h. 7 IV.

a
b
c
d
e
f
g
h

The percentage of chromosomes associated as bivalents at diakinesis was reduced in N_1 populations. There was a wide range in multivalent associations over four in the N_1 populations (Fig. 2a - g). The largest association observed was a chain of 18 (Fig. 2a). The most common associations were those of six, eight, and ten in order of decreasing frequency.

Cytogenetic analysis of microsporocytes at anaphase I showed a higher frequency of irregular anaphase divisions in the N_1 populations as compared to the F_1. The F_1 had 88.5% regular divisions, the one-half-hour treatment 65.8%, and the one-hour treatment 63.2%. The majority of the irregularities involved unequal distribution (Fig. 3a and b) and laggards showing chromatid separation (Fig. 3c). Some bridging or stickiness was observed at anaphase I, but there was no evidence of accompanying fragments.

The percentage of fertility for the F_1 and N_1 is presented in Table 6. Fertility counts represent seed set from cross fertilization between plants in isolated treatment blocks.

In this study there was little difference in survival and general vigor between F_1 and N_1 populations, although a reduction in stainable pollen and percentage of fertility was found in the one-hour treatment of the N_1 population.

The number of culms per plant from the F_1 and N_1 progeny of P.I. 237174, P.I. 251112, and the Swedish varieties Fröde and Brage were counted to obtain a measure of

Fig. 2. Microsporocytes from plants of the M_1 population showing range in multivalent formations over four: a. 5 II, 1 chain of 18; b. 4 II, 1 IV, 1 chain of 16; c. 1 II, 3 IV, 1 chain of 14; d. 6 I, 5 II, 3 IV; e. 6 II, 2 IV, 1 chain of 8; f. 6 II, 1 IV, 1 ring of 12; g. 7 II, 1 IV ("Frying pan" formation), 1 chain of 10.

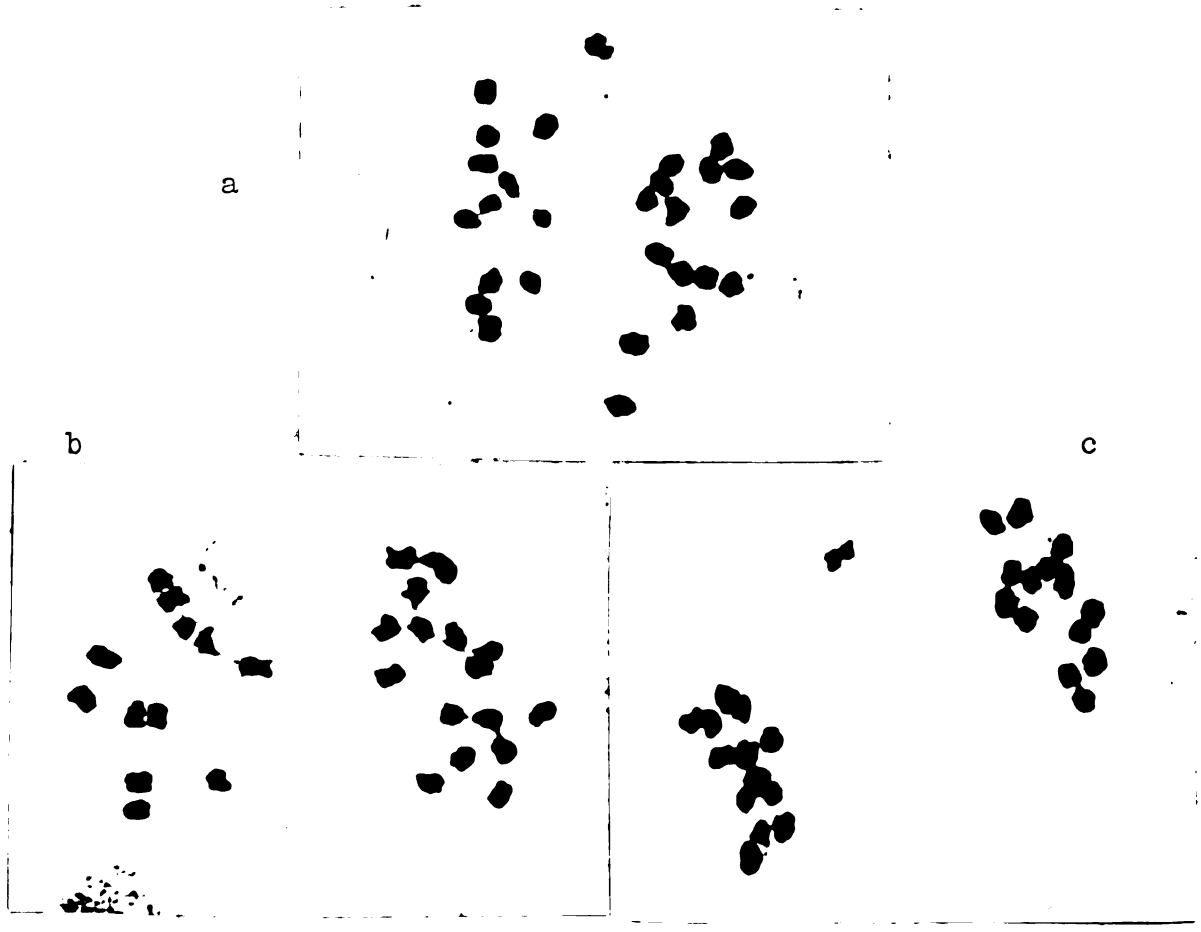


Fig. 3. Microsporocytes from plants of the N_1 population showing irregularities at anaphase I: a. unequal separation 13:15; b. unequal separation 12:16; c. chromatid separation.

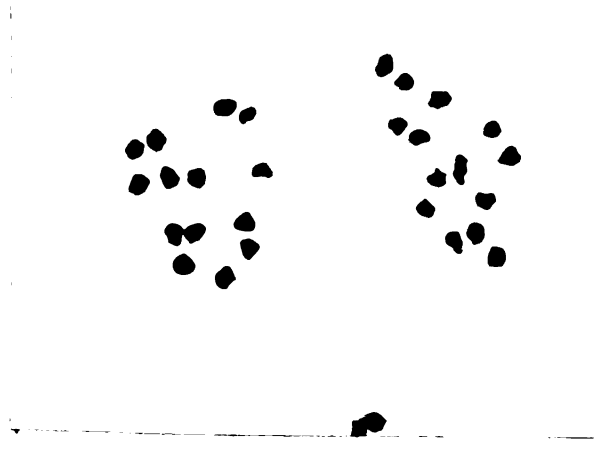


Fig. 4. Normal anaphase I showing equal separation to both poles.

variability for this characteristic. The percentages of plants with under ten culms and the range in culm number per plant are presented in Table 7. All plants were vigorous and had been in the field since June of the previous year, which should have been sufficient for the induction of floral primordia.

TABLE 6. Ranked means of percentage of fertility in the F_1 and N_1

Treatment		½ hr. (N_1)	Control (F_1)	1 hr. (N_1)
Means:	Transformed	35.23	35.15	23.99
	Actual	33.01	34.54	17.53
LSR 1% level				

$\bar{X}$ = 31.46 F value = 32.65** $S\bar{x}$ = 1.13 C.V.% = 24.91

** Significant at the 1 percent level.

The number of culms per plant was used as a measure of the leaf to culm ratio; the higher the number of culms, the lower the leaf to culm ratio. Leafiness is usually associated with high quality forages.

The percentage of germination for the F_2 and N_2 is presented in Table 8. The seed from both the one-fourth and one-half-hour treatments were significantly higher in germination than the control. There was no difference between the control and one-hour treatment.

The percentage of survival for the F_2 and N_2 is presented in Table 9. There was no significant difference in

TABLE 7. Percentage of plants with under ten culms, range in culms per population, for plants from P.I. 237174, P.I. 251112, Fröde, Brage, and a cross 5 X 35-38

Plant No.	Percentage of Plants With Under 10 culms	Range
P.I. 237174		
2.		
Control	46.5	0-108
¼ hr.	60.0	0-79
½ hr.	24.0	0-110
1 hr.	20.7	0-125
3.		
Control	34.8	0-107
¼ hr.	32.7	0-109
½ hr.	23.3	0-116
1 hr.	21.7	0-126
4.		
Control	64.5	0-155
¼ hr.	64.4	0-87
½ hr.	69.4	0-49
1 hr.	55.9	0-119
5.		
Control	42.0	0-128
¼ hr.	32.9	0-145
½ hr.	39.7	0-95
1 hr.	49.4	0-94
Polycross		
Control	51.8	0-123
¼ hr.	62.7	0-97
½ hr.	53.2	0-134
1 hr.	62.1	0-107
Plant 5 X 35-38	0	1-95
Fröde	0	30-134
Brage	0	35-158
P.I. 251112	0	56-175

survival between the control, one-half and one-fourth-hour treatments.

TABLE 8. Ranked means of percentage of germination in the F_2 and N_2

Treatment		¼ hr. (N_2)	½ hr. (N_2)	Control (F_2)	1 hr. (N_2)
Means:	Transformed	78.5	73.0	68.5	68.3
	Actual	94.6	88.5	84.8	84.4
LSR 5% level		———	———	———	

$\bar{X}$ = 71.90 F value = 19.50** S = 8.48 C.V.% = 11.79

** Significant at the 1 percent level.

TABLE 9. Ranked means of percentage of survival in the F_2 and N_2

Treatment		Control (F_2)	½ hr. (N_2)	¼ hr. (N_2)	1 hr. (N_2)
Means:	Transformed	53.52	50.05	49.29	40.14
	Actual	63.60	58.11	56.70	42.52
LSR 5% level		———	———	———	

$\bar{X}$ = 48.83 F value = 14.71** S = 11.52 C.V.% = 23.59

** Significant at the 1 percent level.

The results of the spring vigor rating for the F_2 and N_2 are presented in Table 10. The rating was made on May 10, 1961, at which time differences could be observed readily between plants. At this time three plants were noted in the one-half-hour treatment that were more vigor-

ous than any noted in the control population. These plants were checked cytogenetically for translocations, the results of which are presented later.

TABLE 10. Ranked means of percentage of vigor in the F_2 and N_2

Treatment		Control (F_2)	½ hr. (N_2)	¼ hr. (N_2)	1 hr. (N_2)
Means:	Transformed	29.63	28.75	26.45	21.78
	Actual	25.31	24.01	21.02	14.83
LSR 5% level					

$S\bar{x}$ = 27.07 F value = 27.98** S = 7.87 C.V.% = 29.08

** Significant at the 1 percent level.

Cytogenetic analysis of the N_2 populations revealed a high frequency of plants heterozygous for translocations. Table 11 gives the number of plants analysed in the F_2 and N_2 populations with the number that were heterozygous for translocations. Of the N_2 plants analysed, 57.8% were heterozygous for translocations. The largest association found in N_2 microsporocytes was a chain of 14. Associations of six and eight were most common, although some associations of ten and twelve were observed. There were some microsporocytes observed with univalents (Fig. 2d) and trivalents, but not any more than in the control.

Plants heterozygous for translocations were maintained in the N_2 by cross fertilization within N_1 populations. The high number of N_2 plants heterozygous for trans-

locations was evidence of this.

The control plants analysed from the F_2 had normal bivalent and quadrivalent associations. There were no multivalent associations observed over four.

TABLE 11. Number of plants analysed in the F_2 and N_2 populations with the number of plants heterozygous for translocations

Treatment	Number of Plants Analysed	Number of Plants Heterozygous for Translocations
Control (F_2)	10	0
¼ hr. (N_2)	10	4
½ hr. (N_2)	21	13
1 hr. (N_2)	14	9
Total	55	26

Analyses of 159 anaphase microsporocytes from some N_2 plants gave 94 regular and 65 irregular cells or about 59.1% regular divisions. This was similar to that observed for regular anaphase divisions in the one-half and one-hour treatments of the N_1. Again unequal division and laggards undergoing chromatid segregation were the irregularities observed most frequently.

There was one plant with a chlorophyll deficiency in the N_2 population, visible only for a short period.

The percentage of fertility for the F_2 and N_2 is presented in Table 12. Fertility counts represent seed set from random cross fertilization between and among plants of the F_2 and N_2 populations represented in the randomized blocks.

There were no significant differences between treatments.

TABLE 12. Means of percentage of fertility in the F_2 and N_2

Treatment	Percent		
	Transformed	Actual	
Control (F_2)	32.6	29.0	
¼ hr. (N_2)	37.8	37.6	$\bar{X}$ = 34.83
½ hr. (N_2)	34.0	31.3	F value = 2.23^{NS}
1 hr. (N_2)	35.1	33.1	C.V.% = 25.45

^{NS} Not significant at the 5% level.

The results for the green weights of individual plants from the F_2 and N_2 populations are presented in Table 13.

TABLE 13. Ranked means of the average green weights in grams of individual plants in the F_2 and N_2

Treatment	Control (F_2)	½ hr. (N_2)	¼ hr. (N_2)	1 hr. (N_2)
Means	435.8	419.1	399.4	342.8
LSR 5% level				

$\bar{X}$ = 399.3 F value = 11.59** S = 92.35 C.V.% = 23.13

** Significant at the 1 percent level.

The results for the three largest N_2 plants versus the three largest control plants are given in Table 14. When the t test was applied there was a significant difference at the 20% level. There was no difference in fertility between the three N_2 plants and the average fertility for the F_2 population.

TABLE 14. Plant weights in grams and percentage of fertility for the three heaviest single plants from the irradiated (½ hr.) and control populations in the F_2 and N_2

Plants Heterozygous For Translocations From N_2			Control Plants (F_2)	
Plant No.	Weight	Fertility	Weight	Fertility
(1)	1,204	26.95	1,055	Average
(2)	1,560	29.04	1,092	For Control
(3)	1,192	28.20	1,178	29.0

$t_4 = 1.67$ $t_{.20,4} = 1.53$

All three N_2 plants were heterozygous for translocations. Plant number one (Fig. 5) had microsporocytes with a ring of six (Fig. 6) and an occasional microsporocyte was observed with a ring of six and a ring of eight. This would indicate that at least four translocations were involved if a bivalent and quadrivalent were involved in the ring of six and two quadrivalents in the ring of eight.

Plant number two (Fig. 7) had microsporocytes with chains or rings of eight (Fig. 8a) and ten (Fig. 8b) with some microsporocytes showing both a ring of eight and a chain of ten (Fig. 8c), indicating that at least five translocations were involved. This would be a minimum number if two quadrivalents were associated in the ring of eight, with one bivalent and two quadrivalents associated in the ring of ten. The number of translocations could be higher if the associations originally involved more bivalents. Smaller translocations not forming chiasmata might also have been involved.

Fig. 5. Plant number one with a general view of the F_2 and N_2 populations.

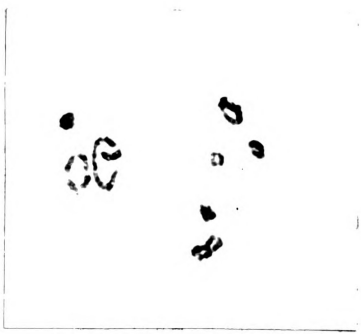
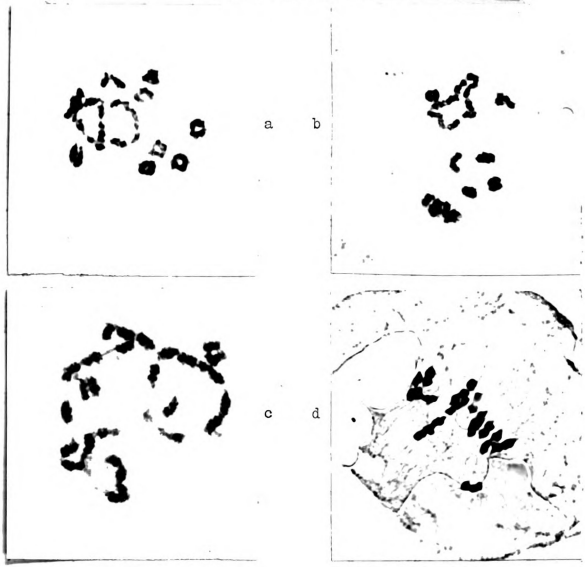


Fig. 6. Microsporocyte from plant number one, showing 7 II, 2 IV, and 1 ring of 6.

Fig. 7. Plant number two heterozygous for translocations.

Fig. 8. Microsporocytes from plant number two: a. 8 II, 1 IV, 1 chain of 8; b. 7 II, 1 ring of 10; c. 2 I, 2 II, 1 IV, 1 ring of 8, 1 chain of 10; d. metaphase showing separation of chain of 8.


a
b
c
d

Associations combining a ring of six and a chain of eight were found in plant number three (Fig. 9), indicating at least three translocations.

Reciprocal crosses were made among these three plants to obtain fertility data as well as seed for further study. The number of seed set per panicle for the crosses and their reciprocals was as follows:

Plant three X two: 29.7

Plant three X one: 72.0

Plant two X one: 48.4

The seed obtained from these crosses will be re-irradiated and grown out for further studies.

The fertility displayed under open pollination and by controlled crosses indicate that the translocations involved do not severely affect seed set.

Self fertility was checked by bagging several panicles on each plant. The number of seed set per panicle was as follows:

Plant one: 2.60

Plant two: 0.08

Plant three: 0.33

There were approximately 1,000 florets on each panicle.

Three additional, less vigorous plants with large translocations were retained for study. One had a chain of eight in about one-half of the microsporocytes examined, with a few showing two chains of eight. The second had a chain of six or a chain of eight in each microsporocyte, with one show-

Fig. 9. Plant number three which had microsporocytes with a ring of 6 and a chain of 3.

ing a chain of 14. The third plant had a ring of six in over half of the microsporocytes examined.

Five plants showing the most vigor in the F_2 were also kept for further study. These three populations, one consisting of three vigorous translocation types, a second with three less vigorous translocation types, and a third with five F_2 plants, were placed in small isolated polycross nurseries for seed increase within each group.

PART TWO

POLYPLOID STUDIES IN *LOLIUM*

MATERIALS AND METHODS

Superior plants of *Lolium multiflorum* (from P.I. 241912, 238937, 238886) and *Lolium perenne* (P.I. 234442) were selected on the basis of vigor, rust resistance, and non-flowering habit in the first year of growth. During the winter of 1959-60, reciprocal interspecific crosses were made between eight *L. multiflorum* and five *L. perenne* plants in the greenhouse by isolating individual spikes in glassine bags and changing the bags every other day. Adequate seed was produced from 35 hybrid crosses. Intraspecific crosses were made at the same time.

Interspecific reciprocal crosses were made between a second group of superior plants (*L. multiflorum*: P.I. 238885, 241912, and 238937; *L. perenne*: P.I. 234442, 189154, 201185, 201186, and 201187) during the winter of 1960-61, in the greenhouse, by isolating the spikes of one *L. multiflorum* and one *L. perenne* clone under polyethylene bags supported on wire cages. This provided an easy method of isolation, while at the same time allowing for the production of a greater number of seed. Seed was obtained from 56 reciprocal crosses. Seed from this group were treated only

with colchicine.

Seed obtained from crosses (both inter and intra-specific) made during the 1959-60 season were given two different treatments. Treatment one (T_1) consisted of colchicine only, while seed for treatment two (T_2) received irradiation at a flux of 7.7×10^8 N/cm^2/sec for one-half-hour, plus colchicine.

Germinated seed (roots 1 - 6 mm in length) were immersed in a .15% aqueous solution of colchicine for two hours, maintained at 30° C. After treatment, the seedlings were washed in cold water for ten minutes, after which they were placed on blotters in petri dishes. They were allowed to develop under controlled conditions (16 hours at 20° C., 8 hours at 30° C., with an eight hour light period) for two weeks prior to transplanting.

Surviving seedlings were transplanted into two-inch peat pots, and attained 15 to 20 leaves before vernalization. The plants were subsequently transplanted to the field. During November 1960, plants showing doubled sectors (Fig. 10) were brought into the greenhouse and divided into diploid and polyploid sectors. Prior to flowering, microsporocytes from each clone were analysed cytogenetically to insure diploid and tetraploid clones. If two culms were found showing tetraploid microsporocytes, that clone was placed in isolation for cross fertilization with other tetraploids. All other clones were allowed to intercross in an isolated section for diploids.

Fig. 10. Plant showing tetraploid sector. This was one of the methods used to separate tetraploid from diploid tissue, prior to cytogenetic analysis.

Seed obtained from the corresponding diploid and tetraploid clones was germinated and the seedlings placed in a field design for a comparison of the two groups.

The Lolium multiflorum design was made up of two tetraploids and a composite diploid strain from the same cross. Each of 16 replications consisted of three randomized plots. Each plot had 30 plants, planted on three-foot centers in rows three feet apart. Thus, the plots were 9 x 30 feet, three rows per plot, 10 plants per row.

The hybrid design was made up of six tetraploids and their corresponding diploids. There were eight replications with plots as described above.

Water and fertilizer (400# per acre of 15-15-15) were applied at the time of planting.

Individual plant dry weight in grams was taken from the ten center plants of each plot. Each plant was placed in a kraft paper bag and air dried for one week prior to weighing.

General growth characteristics and disease reactions were noted throughout the summer.

Rust infection readings in the L. multiflorum populations were based on the presence or absence of rust spores, and converted to a percentage of rust free plants per plot. Percentages were transformed by the arcsin method.

Statistical differences between means were determined by Duncan's (1955) New Multiple Range Test.

Microsporocyte analyses were made from spikes fixed in Newcomer's solution. Observations were made from temporary propionic-carmine slides.

RESULTS

The results deal mostly with the separation and development of tetraploid populations. Two generations of crossing and selection are necessary to obtain a completely tetraploid population since the original colchicine treated plants were mixoploid, capable of intercrossing and producing tetraploid, triploid, and diploid plants. A majority of plants in the tetraploid population so established were tetraploid, while the diploid populations contained a few triploid and tetraploid plants.

Two completely doubled Lolium multiflorum plants and portions of three additional plants free of rust were obtained from 140 treated seed. Three were from T_1 and two from T_2. When intercrossed, one plant from each treatment produced sufficient seed to be propagated from the field experiment. Seed obtained from the two other plants was grown out in progeny rows.

The percentage of plants flowering in the first year was as follows:

Diploid population:	30.5
Tetraploid population:	5.3

Also, there was less tendency for the tetraploid plants to flower after clipping as compared to the diploids. This was an important advantage since diploid L. multiflorum continued

to produce numerous culms after clipping resulting in a high culm to leaf ratio.

Table 15 presents the percentage of plants with rust lesions. The majority of infected diploid plants were severely injured, resulting in the death of most leaves. The tetraploid plants were more tolerant, with no leaves being completely killed. Most tetraploid plants had leaves with lesions well separated and little or no coalescence occurring.

TABLE 15. Ranked means of percentage of plants free of rust lesions in diploid and tetraploid Lolium multiflorum populations

Treatment		701(4n, T_1)	704(4n, T_2)	601(2n)
Means:	Transformed	67.5	55.1	48.0
	Actual	83.5	66.7	55.2
LSR 1% level				

$\bar{X}$ = 56.9 F value = 30.5** $S\bar{x}$ = 1.78 C.V.% = 12.53

** Significant at the 1 percent level

The results of metaphase I analyses of three tetraploid and five diploid plants are presented in Table 16.

Chromosome numbers were determined for ten plants from the tetraploid populations, of which one was triploid, one was diploid, and eight were tetraploid. All diploid plants from the diploid population had chromosome numbers of 2n=14. This was expected since they were derived from seed of the same cross as the induced tetraploid population, which

had not been treated with colchicine.

TABLE 16. Types of pairing in diploid and tetraploid Lolium multiflorum

Cross	Code	Treatment	Average Associations per PMC I	II	III	IV
Tetraploid						
	703	T_2		8.40		2.80
	701	T_1	3.13	6.63	.13	2.63
	704	T_2	1.50	8.75		2.50
		average/plant	1.28	7.93	.04	2.64
Diploid						
	701	T_1		7		
	601	Control		7		
	601	Control	.29	6.86		
	601	Control	.29	6.86		
	601	Control		7		
		average/plant	.12	6.94		

The results of microsporocyte analysis of anaphase I divisions are listed below as the percentage of regular divisions:

Diploid: 92.3

Tetraploid: 54.2

Chromatid segregation, laggards (Fig. 11), and unequal distribution of chromosomes were the most frequently observed irregularities.

Average individual plant dry weights are presented in Table 17. Approximately 10% of the tetraploid populations

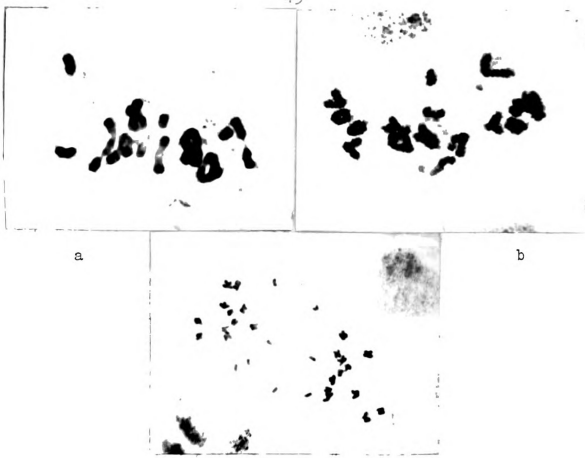


Fig. 11. Microsporocytes from tetraploid Lolium multiflorum plants: a. 5 I, 3 II, 1 III, 2 IV, 1 ring of 6; b. 2 I, 9 II, 2 IV; c. anaphase showing chromatid segregation and laggards.

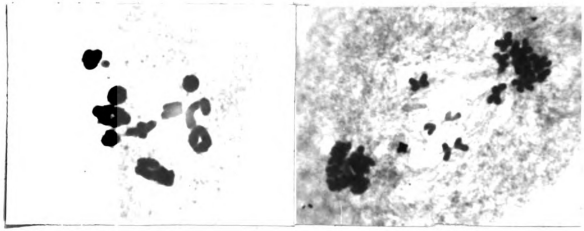


Fig. 12. Microsporocytes from a monosomic (2n=27) hybrid plant: a. 1 I, 7 II, 3 IV; b. chromatid segregation at anaphase I.

consisted of plants with 10 to 15 leaves that were thick, tough, and 4 to 10 inches in length; whereas, such plants were not found in the diploid population.

TABLE 17. Ranked means of individual plant dry weights in grams in diploid and tetraploid Lolium multiflorum populations

Treatment		601(2n)	704(4n, T_2)	701(4n, T_1)
Means		114.1	91.5	90.9
LSR	1% level	———	———	
	5% level	———		———

$\bar{X}$ = 98.8 F value = 5.35** $S\bar{x}$ = 5.71 C.V.% = 23.13

** Significant at the 5 percent level.

Rust was not a severe problem in the hybrid populations. Only a few scattered plants had rust lesions, with no plants having severe infections.

There were very few plants flowering in the diploid and tetraploid L. multiflorum X L. perenne hybrid populations in the first year of growth. Plants in lines 506 and 827 (paired diploid and tetraploid lines) flowered heaviest with approximately two plants flowering per plot in line 827 and four plants in line 506. The other lines had none or one or two plants flowering per plot. Lolium perenne (2n=14) introductions observed at this station have not flowered in the first year of growth, while at least 30 to 100% of the plants in Lolium multiflorum (2n=14) introductions produced spikes

in the first year. It is evident that flowering has been reduced in the diploid and tetraploid hybrids below that observed in L. multiflorum populations in the first year of growth.

The average individual plant dry weights in grams for the hybrid populations are given in Table 18. There were more differences between lines than between corresponding diploid or tetraploid populations. In both T_2 populations the diploids outyielded the tetraploids, while in the T_1 populations the tetraploids outyielded the diploids in three out of four populations. These differences were not significant, but indicate a trend deserving of further study.

The results of cytogenetic analysis at metaphase I in the hybrid populations are presented in Table 19. Chromosome numbers were determined for 21 plants from the hybrid tetraploid populations, of which eleven were tetraploids (Fig. 15), one triploid (Fig. 13), one monosomic (Fig. 12) (2n=27), and eight diploids (Fig. 14). Chromosome numbers were determined on six plants from the diploid populations giving four diploids, one triploid, and one tetraploid. This resulted from outcrossing among mixoploid plants.

The results of microsporocyte analysis of anaphase I divisions are listed below as the percentage of regular divisions.

Diploid: 77.8

Tetraploid: 35.0

Plants have been selected from the second group of

hybrid plants treated with colchicine which show evidence of doubled sectors, but these have not been confirmed by cytogenetic studies. Most of the lines in this group of crosses were free of rust lesions during 1961.

TABLE 18. Ranked means of dry weight in grams of individual plants from diploid and tetraploid hybrid Lolium multiflorum X L. perenne populations

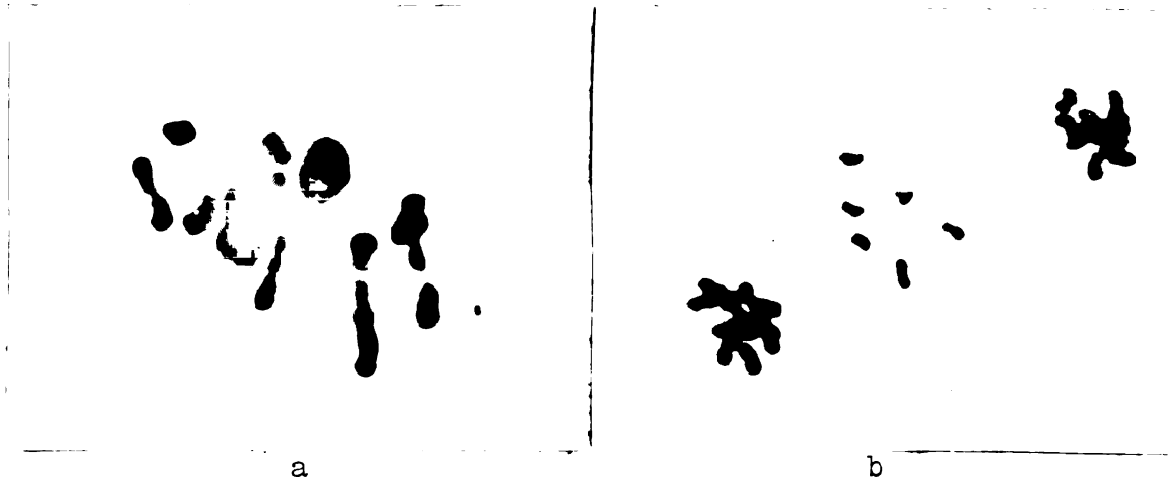
Cross	Code[1]	Treatment	$\bar{X}$	LSR .01	LSR .05
9 X 3	506	T_2	167.6		
3 X 11	828	T_1	154.5		
9 X 3	827	T_2	149.4		
3 X 11	502	T_1	124.7		
7 X 9(a)	507	T_2	111.5		
7 X 9(b)	814	T_1	110.0		
7 X 12	505	T_1	100.5		
7 X 9(a)	829	T_2	93.9		
7 X 12	816	T_1	92.2		
7 X 9(b)	504	T_1	90.7		
2 X 10	802	T_1	87.2		
2 X 10	501	T_1	72.5		

$\bar{X}$ = 112.9 F value = 7.62** $S\bar{x}$ = 8.89 C.V.% = 27.26

** Significant at the 1 percent level.
[1]Code. 5 = diploid population 8 = tetraploid population

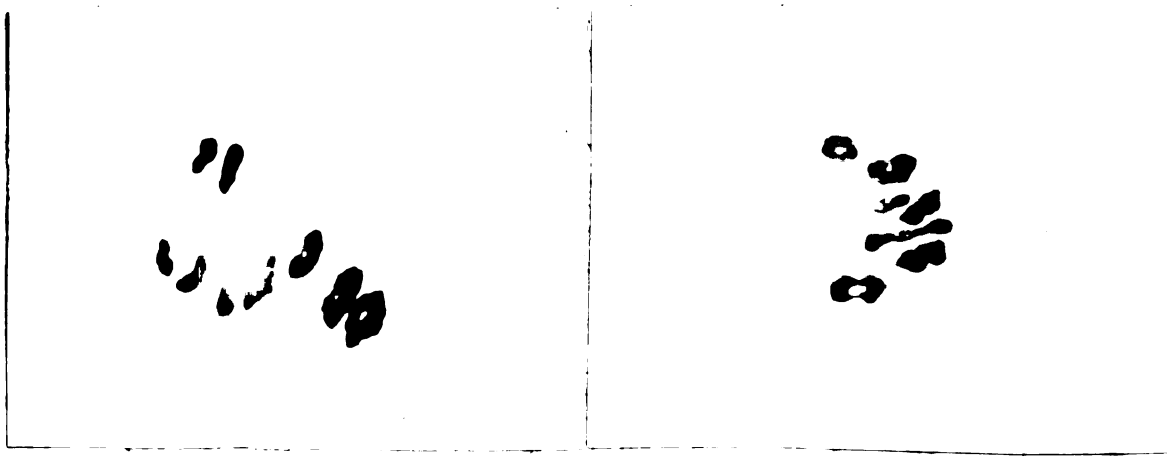
TABLE 19. Types of pairing in diploid and tetraploid hybrids of *Lolium multiflorum* X *L. perenne*

Cross	Code	Treatment	Average Associations per PMC			
			I	II	III	IV
Tetraploid						
	813	T_1		7.07		3.47
	809(monosomic)	T_1	1.0	7.0		3.0
	813	T_1	.50	8.17		2.83
	827	T_2	.33	10.17		1.83
	827	T_2	.94	6.94		3.35
	506	T_2	1.18	8.36		2.46
	827	T_2		7.33		3.33
	801	T_1	.86	9.0		2.0
	average/plant		.60	8.01		2.73
Diploid						
	805	T_1	.64	6.73		
	501	T_1	.13	6.39		.25
	811	T_1		7.0		
	501	T_1		7.0		
	827	T_2		7.0		
	506	T_2	.11	6.78		
	average/plant		.16	6.82		.04



a b

Fig. 13. Microsporocytes from a triploid hybrid plant: a. 3 I, 7 II, 1 IV; b. anaphase.



a b

Fig. 14. Microsporocytes from diploid hybrid plants: a. 4 I, 5 II; b. 7 II.

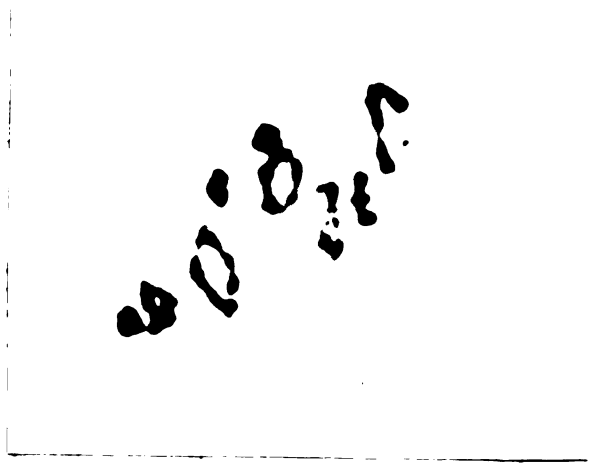


Fig. 15. Normal metaphase from a tetraploid hybrid plant; 6 II, 4 IV.

DISCUSSION

Dactylis. Significant differences were found between the F_2 and N_2 populations for certain characteristics. These differences were not necessarily evident on a plant-to-plant basis, but showed up between populations as a whole. This can be explained partly when it is considered that these were cross fertilized populations with little or no self fertilization occurring and that Dactylis glomerata is an autotetraploid (Myers, 1947, and McCollum, 1958) which exhibits tetrasomic inheritance (Myers, 1941). These factors combined with the fact that most mutations act as recessives genetically would account for the absence of any observable morphological changes attributable to point mutations. Also, this makes it doubtful that homozygous translocations would be found in large numbers in the N_2.

Another effect of cross fertilization was the maintenance of translocations as "floaters" in the population. As the interchanges float in the population they may form combinations showing heterosis with high survival value. In natural populations, especially those which are perennial, where asexual propagation accompanies sexual, floaters are maintained when they are associated critically with survival. If this were the case, they might eventually predominate in the population, depending on the breeding system. In con-

trolled systems it may be possible to direct the formation of valuable structural hybrid populations if plants are available which are heterozygous for a number of translocations.

The generally significant differences observed between the one-hour treatment and the rest of the F and N populations indicated that an effective range in treatments was obtained. The one-hour treatment gave a substantial reduction in survival, vigor, and individual plant weights when compared with the control, one-fourth, or one-half-hour treatments. Cytogenetic analyses in the F_1 and N_1 populations indicated that a significantly higher translocation rate took place from the one-hour treatment. Undoubtedly this higher rate was accompanied by a greater number of deletions, duplications, and other chromosomal aberrations not visible under the light microscope. A combination of these chromosomal changes in the N_2 obviously resulted in a generally weaker population from the one-hour treatment.

It is important to note that fertility was not reduced in the N_2 populations. Good seed set was obtained even when controlled crosses were made between plants heterozygous for a number of translocations. This may be a result of the duplication of chromatin material in tetraploid orchardgrass. Zygotes formed from gametes carrying chromosomal aberrations may function, reducing vigor in populations derived from plants with large numbers of chromosomal aberrations as was observed in the N_2 one-hour treatment.

The combination of large induced translocations in

N_2 plants which were more vigorous than any plants observed in the F_2 populations was perhaps the most significant contribution of this study. The following desirable characteristics were associated with vigor in these plants:

(1). Good fertility

(2). Freedom from foliar diseases

(3). Fairly low silica deposits on leaf margins and leaf sheaths

(4). High leaf to culm ratio

Most previously reported increases in yield (only kernel weight) due to irradiation have been attributed to accumulation of useful mutations as a result of selection for three to six generations. Some yield mutations have been associated with deficiencies and duplications (Mac Key, 1954), while for others no cytogenetic evidence has been presented associating them with chromosomal aberrations.

The increase in vigor for certain plants observed in this study has been associated with chromosomal translocations through cytogenetic studies. The only rearrangements observed under the light microscope in this study were large heterozygous translocations. However, in a number of instances it has been observed through genetic and cytogenetic studies, that large translocations are usually accompanied by duplications, deficiencies, and smaller translocations which fail to form chiasmata (see reviews by Burnham, 1956, and Elliott, 1958). Menzel and Brown (1954) reported genetic evidence for duplications and deficiencies accompanying translocation asso-

ciations at metaphase I in Gossypium hirsutum. Thus, along with the large translocations observed, other chromosomal aberrations undoubtedly were present in these plants.

This increased vigor may be due to the breakage of certain linkage groups where inhibiting genes for growth and vigor are eliminated or brought into new interchange relationships. Also, position effects and mutations associated with chromatin breakage and rearrangements may give new combinations affecting the metabolic activities of the plant.

Evidently large chromosomal changes are not necessarily detrimental, but may enhance vigor, especially in polyploids such as Dactylis glomerata.

The use that can be made of vigor induced through translocation is of importance. If this were a vegetatively propagated plant, immediate use could be made of it, especially when coupled with other desirable characteristics. More progress might be made through irradiation coupled with two or three generations of cross pollination and selection in vegetatively propagated plants, allowing for recombination between genomes with new rearrangements that might result in greater vigor associated with desirable characteristics. This would be in contrast to many reports where vegetative cuttings have been irradiated with the goal of obtaining useful mutations which could then be propagated.

Induced translocations may be of value in improving plants which are propagated by seed. Efforts have been made in orchardgrass improvement programs, to use inbreeding in

obtaining useful homozygous plants with good recombining ability for parental clones. This has met with varying degrees of success by various workers, with Hanson et al. (1952) finding it an effective tool, while Kalton et al. (1952) dismissed it as not being of value. Of ten orchardgrass varieties released for certification, none have been developed through the use of inbreeding (Hanson, 1957).

During the initial development of a new variety, a wide base in genetic variation is desirable. After desirable characteristics have been recombined in parental clones, methods must be used to reduce the amount of segregation occurring when synthetic varieties are increased. In the development of synthetic forage grass varieties the main method of breeding to date has been that of mass selection with the polycross technique becoming more popular. Although some improvement has been made with these methods, progress has been slow.

New breeding techniques that may be used to supplement present breeding procedures to attain greater uniformity are:

(1). Translocation-induced structural hybridity

(2). Breeding and selecting fairly homozygous diploid clones, and resynthesis of polyploids from these diploids. The induced polyploids may be used to transfer desired genes to other polyploid parental clones. They might possibly be used directly as parental clones. Diploid source materials may be obtained from ancestral relatives or from

polyhaploids which appear occasionally in polyploid populations.

In populations similar to the one under study there exists adequate variation for selection of desirable characteristics. However, clones which show good recombining ability rarely appear or are difficult to identify. Irradiation might be used either to produce more variation through point mutations or induced translocations. In this way, vigor might be enhanced, combined with other desirable characteristics, and maintained by decreasing recombination.

The availability of several vigorous orchardgrass plants having between them a number of induced translocations gives rise to the possibility of creating structural hybridity in a closed outcrossing population, similar to that found in Oenothera, Rhoeo, Campanula, and other species. Burnham (1946) has proposed such a system for corn and barley.

Following is a proposal by which such a population could be created in orchardgrass using materials now available.

(1). Make reciprocal crosses among the three translocation heterozygotes to accumulate the existing translocations. This would take at least two generations as biparental pairing would have to be practiced. After the first generation, selection should be made for plants heterozygous for a number of translocations associated with vigor and other desirable plant characteristics.

(2). Another possibility is the open pollination in small isolated polycross nurseries of the selected popula-

tions (vigorous and intermediate translocation heterozygotes, and the control population) to allow free exchange of translocations. Each system should be kept closed, allowing an accumulation of translocations within each group.

(3). Irradiate seed obtained by the above methods with neutrons at a flux of 7.7×10^8 N/cm^2/sec for one-half hour. The induction of new translocations in the population should result in the possibility of creating larger ring and chain formations. Also, plants heterozygous for only one or two translocations could segregate out gametes with a normal chromosome complement. Thus, there is the possibility that plants with completely normal chromosomal complements could be produced. However, since the plants are polyploid, cross fertilized, and heterozygous for translocations, the chance of recovering "normal" plants will be greatly reduced, especially with another cycle of irradiation.

(4). Let cross pollination take place between the second cycle irradiated plants and grow out the next generation selecting the most vigorous types for cytogenetical analysis.

Ideally, permanent structural heterozygotes would have all the chromosomes in one large ring, and alternate disjunction associated with a balanced lethal system. Balanced lethal systems would not necessarily be derived at the same time as large chromosome rings. Balanced lethal systems are necessary for the elimination of structural homozygotes. In this manner a population could be obtained having progeny

with uniform characteristics.

This will happen in a selfing or inbred population but would be difficult to attain in cross pollinated populations as was shown by Darlington and LaCour (1950) in Campanula persicifolia. However, this does not preclude the possibility of reducing recombination and segregation in cross pollinated populations. Translocation heterozygotes should result in the formation of smaller rings and chains, reducing considerably the recombination of whole chromosomes.

Also crossing over, and consequently, genetic recombination should be reduced in the regions of the translocations. This should maintain any increased vigor due to the breakage of linkage groups, position effects, or other changes that might have occurred affecting metabolic pathways.

The proposal made above has been initiated. Steps have been taken to carry out a program to see if a structural hybrid system can be obtained in orchardgrass which would result in increased uniformity.

Lolium. Higher rust resistance observed in the tetraploids was accomplished in one cycle, whereas, the diploids had been through two selection cycles for rust resistance. The higher resistance may be due to the additivity of genes involving the interactions of genes whose individual effects may be of minor importance. The inheritance of rust resistance in forages has not received intensive study, however, and little is known concerning its inheritance.

Rust resistance combined with other desirable characteristics of selected tetraploid plants would have considerable value in the production of aftermath growth where susceptibility in the diploids could result in a reduced yield or deterioration in quality.

The high individual dry weight yields obtained from the diploid and tetraploid Lolium multiflorum X Lolium perenne hybrids combined with rust resistance and lack of flowering in the first year of production indicates that the desirable characteristics of the two species have been combined. Only further breeding and selection will tell whether greater progress can be made at the diploid or tetraploid levels.

Certainly there are two approaches to the problem, and as Wit (1954) has pointed out, progress in breeding through polyploidization cannot be expected without the use of other plant breeding techniques. In fact, he has based his program on the accumulation of diploid ryegrasses including types from various origins, doubling, then crossing and selecting at the tetraploid level.

It is too early to tell if winter hardiness has been increased or if a greater margin for selection of this character exists at the tetraploid level. Some progress has been made for this characteristic at this station at the diploid level. However, there is hope of increasing winter hardiness through selection in tetraploids as has been reported in the literature.

Analysis of metaphase and anaphase I microsporocytes

indicate that a high percentage of meiotic irregularities accompanied polyploidization. This is a phenomenon that has been observed in many artificially induced autopolyploids. In fact, Myers (1945) reported that univalents in 224 metaphase I microsporocytes of tetraploid perennial ryegrass were considerably higher than in the diploids, varying from 17.3 to 51.8%. He reported an average quadrivalent frequency of 3.96 per microsporocyte.

The close agreement between autotetraploid Lolium multiflorum and the hybrids of L. multiflorum X L. perenne in various meiotic configurations suggests that cytogenetically the two species do not have many chromosomal differences. Saura (1951) concluded that there were greater differences within groups of diploid hybrid plants of the two species than between groups of plants.

Plants derived from the first generation after doubling were not as a population superior to diploid types. It was not expected that the first doubled plants would be superior to their diploid counterparts. Therefore, introductions were obtained representing wide genetic sources of origin, with the expectation that hybridizing and doubling would result in tetraploids which could be crossed with other induced tetraploids.

There were plants in the tetraploid populations with characteristics superior to those of the diploid. Further crossing and selection of superior types from wide genetic sources might result in the development of superior tetra-

ploid populations.

Workers in the past have based their studies on doubled plants derived from a narrow source of germplasm. Wit (1954), referring to polyploid studies made by Myers and Shalygen in ryegrass, pointed out that their studies were based on a limited amount of initial material, presumably coming from one local strain. He stated that condemnation of induced polyploidy is a mistake without first assembling a large number of genotypes, doubling, and selecting at the tetraploid level over several generations under various agricultural environments.

Irradiation was used to induce translocations between genomes of Lolium perenne and L. multiflorum. The possibility exists of an exchange of chromosomal parts, which might give increased vigor. If this increased vigor is due to heterozygosity per se, then doubling should give permanent fixation of heterosis.

Natural populations through introgression of isolated species have given rise to hybrids with increased survival value. Isolation of these species was probably originally a result of chromosomal aberrations, different maturity, environmental conditions, and other barriers. Polyploidization of these types may give permanent fixation of those combinations which may show a high survival value for the ecological situation in which they developed. It may also reduce reproductive barriers.

Many native perennial polyploid grasses show high

survival value, but lack vigor. Polyploids are considered more tolerant to high elevations and changing climatic conditions than diploids. Thus polyploidization has an application in the development of low temperature tolerances in grasses. In developing new varieties compromises must be made between vigor, seed set, maturity, and survival. A perennial forage grass must persist in the intended environment of use.

CONCLUSIONS

An attempt was made to apply techniques of polyploidization and structural hybridity to practical plant breeding. Radiation-induced translocations and polyploidization offer the breeder potentially useful tools in association with techniques already in use.

Irradiation in Dactylis resulted in a wide range of translocations, as measured by the number of multivalent associations of more than four at diakinesis. One-half-hour of irradiation was more efficient than the one-fourth or one-hour treatments in the induction and survival of translocations. The morphological or fertility characteristics of the N_2 population (one-half-hour treatment) were not affected.

A breeding system for polyploid forage grasses, based on structural hybridity, has been proposed.

Greater rust resistance was found in induced tetraploid Lolium multiflorum populations over corresponding diploid populations. Some tetraploid plants were more vigorous than any found in the diploid populations.

The vigor of Lolium multiflorum was combined with the rust resistance of L. perenne in diploid and tetraploid hybrids. Tetraploid hybrids in the first generation were not superior to corresponding diploid hybrid populations. There were more differences between the original clonal lines than

between the corresponding diploid and tetraploid populations.

Induced tetraploid populations in ryegrass were not superior to the diploid populations. Several cycles of crossing and selection among induced tetraploids derived from wide genetic sources may give superior tetraploid populations.

LITERATURE CITED

Andersson, G. and Olsson, G. 1954. Svalöf's Primex White Mustard - a market variety selected in X-ray treated material. Acta Agriculturae Scandinavica 4:574-577.

Burnham, C. R. 1946. An "Oenothera" or multiple translocation method of establishing homozygous lines. Journal of American Society of Agronomy 38:702-707.

_____. 1956. Chromosomal interchanges in plants. The Botanical Review 22:419-552.

Caldecott, R. S. 1955. The effects of X-rays, 2 MEV electrons, thermal neutrons and fast neutrons on dormant seeds of barley. New York Academy of Science Annals 59:514-535.

Carnahan, H. L. and Hill, Helen D. 1961. Cytology and genetics of forage grasses. The Botanical Review 27:1-162.

Cleland, Ralph E. 1950. Studies in Oenothera Cytogenetics and Phylogeny. Indiana University Publications. Science Series No. 16.

Corkill, L. 1945. Short-rotation ryegrass. Its breeding and characteristics. New Zealand Journal of Agriculture 71:465-70.

D'Amato, F., Avanzi, S., Moschini, E., and Scarascia, G. T. 1958. Primi risultati Italiani sugli effectti di radiojioni in piante agrarie. (The first Italian results on the effects of ionizing radiations on agricultural plants.) ATT. V Rassegna internaz. elettron. nuclear. Roma 1958: pp. 26. Abstracted from Plant Breeding Abstracts 29: no. 2389.

Darlington, C. D. and Gairdner, A. E. 1937. The variation system in Campanula persicifolia. Journal of Genetics 35:97-128.

_____ and LaCour, L. F. 1950. Hybridity selection in Campanula. Heredity 4:217-248.

De Roo, R. 1959. La selection polyploide (polyploid breeding). Rev. Agric., Brux. 12:603-05. Abstracted

from Plant Breeding Abstracts 30: no. 2741.

Desroches, R. 1958. Le ray-grass Italien "la meilleure et la pire" des graminées fourragères. (Italian ryegrass, "the best and the worst" of the forage grasses.) Bull. Engrais 1958: Supple.: 16-21. Abstracted from Plant Breeding Abstracts 29: no. 1618.

Duncan, David B. 1955. Multiple range and multiple F. tests. Biometrics 11:1-42.

_____. 1957. Multiple range tests for correlated and heteroscedastic means. Biometrics 13:164-176.

Elliott, F. C. 1958. Plant Breeding and Cytogenetics. McGraw-Hill Book Company, Inc.

Gaul, Horst. 1958. Present aspects of induced mutations in plant breeding. Euphytica 7:275-289.

Gelin, Olov E. 1954. X-ray mutants in peas and vetches. Acta Agriculturae Scandinavica 4:558-568.

Gregory, W. C. 1955. X-ray breeding of peanuts (Arachis hypogaea L.). Agronomy Journal 47:396-399.

_____. 1956. Induction of useful mutations in the peanut. Brookhaven Symposium in Biology 9:177-190.

Hanson, A. A. 1959. Grass Varieties in the United States. Agriculture Handbook No. 170. Agricultural Research Service, United States Department of Agriculture.

_____, Myers, W. M., and Garber, R. J. 1952. General combining ability of orchardgrass selections and their I_4 progenies. Agronomy Journal 44:84-7.

_____ and Carnahan, H. L. 1956. Breeding Perennial Forage Grasses. Technical Bulletin No. 1145. Agricultural Research Service, United States Department of Agriculture.

Harter, H. Leon. 1960. Critical values for Duncan's new multiple range test. Biometrics 16:671-685.

Hertzsch, W. 1959. Beobachtungen an kunstlich hergestellen polyploiden futterpflanzen. 3. Welsches weidelgras (Lolium multiflorum). Zeitschrieft Für Pflanzenzuchtung. 41:271-93.

Humphrey, L. M. 1954. Effects of neutron irradiation on soybeans. II. Soybean Digest 14:18-19.

Kalton, R. R., Smit, A. G., and Leffel, R. C. 1952. Parent-inbred progeny relationships of selected orchardgrass clones. Agronomy Journal 44:481-6.

Konzak, C. F. 1957. Genetic effects of radiation on higher plants. Quarterly Review of Biology 32:27-45.

Kuchař, F. 1957. Pícnínařské využití mezidruhovéha křížence jílků (agronomic utilization of an intervarietal cross of ryegrass). Za socialist. Zemid 9:541-47. Abstracted from Plant Breeding Abstract 28: no. 4286.

Larter, E. N. and Elliott, F. C. 1956. An evaluation of different ionizing radiations for possible use in the genetic transfer of bunt resistance from Agropyron to wheat. Canadian Journal of Botany 34:817-823.

Mac Key, James. 1954. Mutation breeding in polyploid cereals. Acta Agriculturae Scandinavica 4:549.

_____. 1956. Mutation breeding in Europe. Genetics in plant breeding. Brookhaven Symposium in Biology 9:141-156.

McCollum, G. D. 1958. Comparative studies of chromosome pairing in natural and induced tetraploid Dactylis. Chromosoma 9:571-605.

Mehta, R. K. and Swaminathan, M. S. 1957. Studies on induced polyploids in forage crops. 1. Survey of previous work. The Indian Journal of Genetics and Plant Breeding 17:27-57.

Menzel, Margaret Y. and Brown, Meta S. 1954. The tolerance of Gossypium hirsutum for deficiencies and duplications. The American Naturalist 88:407-418.

Mommersteeg, H. 1957. Les Ets H. Mommersteegs Zaadteelt Zaadhandel, sa. (The seed production and trading establishment of H. Mommersteeg Ltd. Courr. Hort. 19:343-46. Abstracted from Plant Breeding Abstract 28: no. 581.

Myers, W. M. 1941. Genetical consequences of the chromosomal behavior in orchardgrass Dactylis glomerata. Journal of the American Society of Agronomy 33:893-900.

_____. 1943. Analysis of variance and covariance of chromosomal association and behavior during meiosis in clones of Dactylis glomerata L. Botanical Gazette 104:541-552.

_____. 1945. Meiosis in autotetraploid Lolium perenne in relation to chromosomal behavior in autotetraploids. Botanical Gazette 106:304-316.

_____. 1947. Cytology and genetics of forage grasses. The Botanical Review 13:316-421.

_____ and Hill, H. D. 1940. Studies of chromosomal association and behavior and occurrence of aneuploidy in autotetraploid grass species, orchardgrass, tall oatgrass, and crested wheatgrass. Botanical Gazette 102:236-55.

_____ and Hill, H. D. 1942. Variations in chromosomal association and behavior during meiosis among plants from open pollinated populations of Dactylis glomerata. Botanical Gazette 104:171-75.

Müntzing, A. 1933. Quadrivalent formation and aneuploidy in Dactylis glomerata. Botaniska Notiser 198-205.

_____. 1937. The effects of chromosomal variation in Dactylis. Hereditas 23:113-235.

Nishimura, Y. and Kurakami, H. 1953. Analysis and synthesis of reciprocal translocations in barley. Journal of Plant Breeding (Jap.) 3:45-47. This article not seen. As quoted from Burnham 1956.

Osborne, T. S. 1957. Past, present and potential uses of radiation in southern plant breeding. Proceedings of Ninth Oak Ridge Regional Symposium "Radiation in Plant Breeding", sponsored by the University of Tennessee in cooperation with the Oak Ridge Institute of Nuclear Studies.

Papa, Kenneth E., Williams, J. H., and Hanway, D. G. 1961. Effectiveness of selection of quantitative characters in the third generation following irradiation of soybean seeds with X-rays and thermal neutrons. Crop Science 1:87-90.

Saura, F. 1951. Comportamiento cariologico de Lolium perenne Linn, Lolium multiflorum Lam y de su F_1. Buenos Aires Universidad nacional Facultad de agronomia y veterinaria Revista 13:90-107.

Sax, K. 1957. Effects of ionizing radiation on chromosomes. Quarterly Review of Biology 32:15-26 (AI).

Smith, H. H. 1958. Radiation in the production of useful mutations. Botanical Review 24:1-24.

Snedecor, George W. 1956. Statistical Methods, Fifth Edition, The Iowa State College Press, Ames Iowa.

Sparrow, A. H. 1957. Cytological changes induced by ionizing radiations and their possible relation to the production of useful mutations in plants. Report Brookhaven Work Conference. Radiation Induced Mutations pp. 76-114.

Stebbins, G. Ledyard Jr. 1950. Variation and Evolution in Plants. Columbia University Press, New York.

_____ and McCollum, G. D. 1955. The induction of genic and chromosomal sterility in a subspecies hybrid of Dactylis by irradiation of pollen. Genetics 40:590.

Swanson, Carl P. 1957. Cytology and Cytogenetics. Prentice-Hall, Inc.

Wit, F. and Speckmann, G. J. 1954. Breeding of tetraploid ryegrasses. European Grassland Conference. Mimeograph.

Wit, F. 1958. Tetraploid Italian ryegrass (Lolium multiflorum). Euphytica 7:47-58.

_____. 1959. Chromosome doubling and the improvement of grasses. Proceedings of the 5th annual meeting of the Italian Society of Agricultural Genetics, Salice terme-Pania 16-18 June 1958. Polyploidy in the improvement of cultivated plants: beet, forage-crop and fruit cultivation. Genet. agr. 11:97-115. Abstracted from Plant Breeding Abstract 30: no. 1301.

Yamashita, K. 1951. Studies on X-ray induced reciprocal translocations in Einkom wheats. III. A newly synthesized ring of 14 chromosomes in a complex heterozygote, Aegilopoides monococcum. Cytologia 16:164-176.

Zijp, M. J. 1960. Some observations on a possible X-ray mutant in Cocksfoot (Dactylis glomerata). Euphytica 9:222-224.

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