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THE ISOLATION AND CYTOGENETICS OF A MONOTELOTRISOMIC SERIES AND ACCESSORY CHROMOSOMES IN BARLEY (HORDEUM VULGARE L.)

by

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To

My Parents

Naima and Ibrahim Sayed

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ABSTRACT

Trisomic lines for each of chromosomes 1, 3, 4, 5, 6 and 7 of barley (Hordeum vulgare, L.) were subjected to mutagens viz., EMS, DES, HA, FUdR and γ-rays with the objective of inducing telocentrics in the extra chromosome. Lethality of DES and EMS on trisomic seeds and sterility caused by HA and γ-rays in meiosis treatments account for failure to recover any telocentrics from these treatments. In contrast, low doses of γ-rays, alone or in combination with FUdR, significantly increased frequencies of telocentrics over the control. All chromosome breaks induced in chromosome 7 were localized at the centromere and the adjacent region of the long arm.

A total of 17 telotrisomics studied represented 11 distinct chromosome arms. Analysis of karyotype, and crosses to translocations and genetic markers, showed these telotrisomics to constitute a series which lacks only short arms of chromosomes 2 and 4 and the long arm of chromosome 7.

The karyotype analysis indicated an arm ratio higher than the standard for chromosome 1 but a lower arm ratio for chromosome 3. Assignment of genetic markers to specific arms of chromosomes 3 and 5 contradicted prior assumptions based on karyotype analysis, indicating that the existing genetic maps of these chromosomes are reversed.

Telotrisomics for long arms resembled their related trisomics whereas those for short arms were virtually indistinguishable from normal diploids. The extra telocentric associated with its homologues in a heteromorphic trivalent in 82% and 77.6% of PMC's at diakinesis and M_I, respectively. Univalent telocentrics divided precociously at A_I, lagged at T_I and T_{II} resulting in a frequency of 26.7% quartets with micronuclei. Comparisons between satellited and non-satellited telocentrics of chromosome 6 suggested that the erratic behavior of the satellited telocentric was due to the presence of the nucleolus and its interference with chiasma formation. Transmission of telocentrics in selfed progenies averaged 31.1% with no obvious transmission through pollen.

Correlations of arm length in relation to chromosome association and transmission were positive and highly significant. Correlation between the frequency of univalents at M_I and quartets with micronuclei (meiotic index) was also positive and highly significant, suggesting that the meiotic index may be used as a convenient measure for chromosome stability. A telocentric shift was detected in the progeny of telotrisomic 2n+2L.

Accessory chromosomes in barley occurred spontaneously among the progeny of trisomics. They are smaller than normal telocentrics with a globular appearance. Their number per cell varied in both somatic and germinal cells. At meiosis, they were positioned at the equatorial plate, divided precociously at A_I and T_I, and lagged and formed micronuclei at the quartet stage. It is assumed that they arose through misdivision from normal chromosomes resulting in loss of their pairing arms.

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GENERAL INTRODUCTION

Chromosome engineering is a very fruitful technique for plant improvement and is extensively used in breeding programs. Interspecific and intergeneric hybridization, induced ploidy and aneuploidy are good examples of that use.

Although a great deal of genetic and cytological work has been conducted with cultivated barley (Hordeum vulgare L. Edmend. Lam.), its diploid nature had limited chromosome engineering in most cases to translocations. Tsuchiya (1960) established the first complete trisomic series in barley. Therefore, it was used to assign the seven linkage groups to the individual chromosomes. However, trisomic analysis, has not been capable of establishing the relationship between the genetic and cytological map with regard to the centromere position. It seems that telocentrics are the only suitable method to achieve such a purpose. A telocentric chromosome is a single chromosome arm with a terminal centromere. It may replace a normal homologue in polyploids (monotelosomics) or may exist in addition to the normal disomic complement (monotelotrisomics). Since deficiencies or deletions even for small segments are generally lethal in diploids, only telotrisomics are tolerated.

Telotrisomics are very useful in the study of genetic activity of each chromosome arm as well as for linkage analysis and for locating the centromere. Since telotrisomics carry only an extra chromosome arm, they are more vigorous and fertile than their related trisomics (Fedak, 1969).

To date, less than half of the fourteen possible telocentrics have been studied in barley. The aim of this investigation was to establish a telotrisomic series in barley and to this end, trisomics were subjected to different mutagens in order to induce telocentrics in the extra chromosome. Using karyotype analysis the isolated telocentrics then were classified into acrocentrics, true telocentrics and accessory chomosomes. True telotrisomics were identified by means of crossing each to appropriate translocation and genetic marker stocks and studying the meiotic behavior of the F_1-progeny. Each identified telotrisomic was studied in detail morphologically and cytologically, in order to establish the relationship between arm length and chromosome behavior, as well as chromosome behavior and stability. In addition, accessory chromosomes were identified and studied cytologically in somatic and sporogenous cells.

The present thesis is divided into the following sections:

Section I: Induction of telocentrics.

Section II: Cytogenetics of telotrisomics.

Section III: Cytogenetics of accessory chromosomes.

SECTION - I

INDUCTION OF TELOCENTRICS

INTRODUCTION

Although trisomics have been utilized extensively in genetic studies of plants, in some species (e.g. Hordeum vulgare L.) certain difficulties have been encountered in their use. Among these characteristics are poor growth of trisomics relative to normal diploids and low fertility and infrequent transmission of the extra chromosome. For these reasons attention has been drawn to the use of telotrisomics which in comparison with trisomics are more vigorous and fertile.

Telotrisomics involve only an additional chromosome arm rather than a whole chromosome and thereby provide an opportunity to map the position of the centromere relative to known genes located on each arm. To-date the position of the centromere on most of the seven linkage groups of barley has not been clearly demonstrated.

Although telotrisomics in barley occur spontaneously, they do so at a frequency too low for practical purposes (Tsuchiya, 1971; Yu, 1968). The observations of some workers (Ramage et al., 1961; Hagberg et al., 1963) that certain induced translocations involved breaks within centromeres suggest that mutagenic treatments may be an effective method to produce telocentrics.

In the following section, the results of four experiments are reported, each designed to measure the effects of various mutagenic agents on the induction of telotrisomics of barley (H. vulgare L.).

REVIEW OF LITERATURE

Telocentric Chromosomes

Misdivision. Darlington (1939) considered that centromers of different chromosomes were all alike in their form and behavior. Lima De Faria (1958), could distinguish four chromomeres in pachytene chromosomes of maize, disposed in a longitudinal line within the centromere region. He suggested that the centromere structure is a tandem reverse.

Darlington (1939) and Sears (1952) outlined detailed studies of univalent misdivision in Fritillaria and wheat, respectively. Their observations indicated that univalents may divide transversely (misdivide) at the centromere either during A_I or their division products at A_{II} to give rise to chromosomes with terminal centromeres (telocentrics). Moreover, a single centromere could misdivide into four functional parts. Brown (1958) believed that misdivision of the centromere depends more upon a change in timing of normal centromere behavior than upon centromere structure. Marks (1957) considered four points of break; three of them within the centromere, giving rise to true telocentrics with a centromere either complete or deficient, while the fourth occurs in the adjacent arm resulting in an acrocentric. However, from the genetic viewpoint, recombination within a very short arm would be seriously affected due to the restriction in the frequency of crossing over.

Occurrence. The first observation of a telocentric was made by Huskins (1934) in wheat. He described a heteromorphic bivalent caused

by the loss of approximately one half of a chromosome. Among the progeny of trisomic-5 in maize, Rhoades (1936) isolated a plant with an extra fragment chromosome and identified it as the short arm of chromosome-5. Moreover, in Datura, many plants with one or two extra telocentrics were found in the progeny of trisomics (Blakeslee and Avery, 1938). Smith (1947) found a compensating telotrisomic in the progeny of an irradiated spike of T. monococcum. He assumed that a break occurred within the centromere and one of the resulting arms subsequently formed an isochromosome.

While telocentrics are common in the progeny of wheat aneuploids, they are very seldom found in the progeny of barley trisomics. Tsuchiya (1960) and Yu (1968) observed that about 24% of microsporocytes (PMC's) with an extra chromosome contained a univalent. In 50% of these cells, the univalent was positioned at the equatorial plate and occassionally it misdivided. Therefore, the actual frequency of telocentrics in the progeny of these trisomics was very low (0.17% Tsuchiya, 1971). For this reason it was necessary to explore some artificial methods such as using mutagens to induce telocentrics in barley.

Mutagensis has been one of the most extensively investigated fields and as a result, literature pertaining to this field of research is voluminous. However, several excellent reviews are available including those of Praaken (1959), Gaul (1964), Wallace (1964), and Auerbach (1967) who presented a detailed survey of the physical properties, biological manifestations, methodology and some of the results obtained with mutagenic

treatment of plant species. In addition Nilan and his co-workers (1963, 1964, 1968) discussed the factors which modify mutagenic effects. The following review deals with only certain specific aspects of induced chromosomal aberrations as they pertain to the present study.

Mutagens

Two main groups of mutagens are recognized, physical radiation and chemicals. Although both groups induce similar effects, their mode of action is quite different.

Radiation. The penetration of radiation into material is accompanied by an energy transfer known as excitation. In addition, ionizing radiation is capable of producing ion pairs when they interact with matter. This ionization, as it is called, as well as excitation may cause a direct change in nucleic acids resulting in mutations.

Ultraviolet light, the only form of non-ionizing radiation, does not have the ability to ionize but transfers its energy by excitation. Due to its low penetration ability, its use in plant mutation work is generally limited to pollen irradiation (Fabergé, 1957; Stienitz-Sears and Sears, 1957). The mutagenic influence of ultraviolet rays is highest at wave lengths that show the highest absorption by nucleic acids (Praaken, 1959). Ultraviolet radiation increases point mutations relative to chromosome breaks, and when they occur they tend to rejoin less easily and therefore are likely to produce deficiencies which are of limited values.

In contrast, the deep penetration of ionizing radiations (x-rays, γ-rays and neutrons) causes chromosome breakage. However, their effi-

ciency is dependent on the energy dissipation and/or ion density along the track of the ionizing particles that are ejected in the tissue. Since x-rays and γ-rays dissipate their energy in biological material by the production of the smaller electrons, fast neutrons by ejection of the larger protons, and thermal neutrons to a great extent by emission of the even larger alfa particles, a successively increased ability to break chromosomes is therefore expected from the use of these three mutagens. This was confirmed by Leroy (1968) who found that neutrons had a greater mutagenic efficiency than γ-rays for the same survival of M_1-plants. In contrast to non-ionizing rays, all breaks induced by ionizing radiations have generally a relatively high rejoining ability which favor chromosome rearrangements such as translocations, inversions, and duplications.

Chemicals. A large variety of chemical mutagens are known. Alkylating agents, the most potent chemical mutagens, produce in addition to point mutations all types of chromosome aberrations. Because their genetical effects are very similar to those of ionizing radiation, the term "radiomimetic agents" is often applied to them. The alkylating agents exert their biological effects by alkylation of nucleophilic sites in DNA, and more specifically, they attack the N-7 atom of guanine which is thought as the most important biological action of these agents (Caspersson et al. 1967).

Another important class of chemical mutagens are the base analogs or inhibitors of DNA synthesis. They induce mainly chromosmoal aberrations

consisting of gaps and open breaks which are due to interruptions in DNA replication in cells completing the synthesis phase. Torsions and tensions produced by chromosome coiling at prophase as well as anaphase movement would result in fragmentation of these chromosomes. The fact that chromosome rearrangements are rare or entirely absent among aberrations induced by some base analogs as 5-fluorodeoxyuridine (FUdR) indicate that rejoining of chromosomal breaks is inhibited. Taylor et al. (1962) reported that FUdR binds irreversibly to the enzyme thymidlate synthetase and inhibits thymidlic acid synthesis which is needed for DNA replication. Thus, the mutagen is not only capable of inducing chromosome breaks, but it should also inhibit the rejoining of breaks induced by irradiation. This was accomplished by using concentrations lower than those needed for the production of chromosomal breaks. Irradiation in the presence of FUdR greatly increased the frequency of free fragments accompanied by a decrease in the frequency of chromosomal bridges (Kihlman, 1962; Taylor et al. 1962; Moutschen-Dahmen et al. 1966).

Chromosome breaking agents exert their effects during different stages of the cell cycle. Most radiomimetic agents have only a delayed effect, i.e. chromosome aberrations induced in early interphase cells (Kihlman, 1963). Ionizing radiation induce aberrations at all stages of the cell cycle, i.e. non-delayed and delayed effects. Base analogs have a non-delayed effect resulting in the immediate appearance of chromosomal gaps and open breaks in addition to a delayed effect.

Although chemicals have similar effects to those induced by ionizing radiations, three major differences between these two mutagens are known to exist. These are:

(1) Treatment with chemical results in a deficiency of chromosome rearrangements relative to gene mutations. This deficiency is not due to a shortage in chromosome breaks but to the inhibition of the process of rejoining (annealing) (Auerbach, 1967).

Froese-Gertzen et al. (1964) found that x-rays caused similar decreases in seedlings growth and spike fertility whereas EMS affected fertility more than seedlings growth. The cytological analysis of the treated material showed that reduction in fertility was directly related to the frequency of chromosome aberrations after x-rays treatment. In contrast, the pronounced decrease in fertility after EMS treatment was accompanied by a very low frequency of chromosome abnormalities. These results confirm those of Sato and Gaul (1967) who pointed out that chromosome abnormalities were not considered to be high enough to account for the extreme sterility induced in barley by EMS. They concluded that small deficiencies may be the main cause of sterility in EMS-treated material.

In an attempt to study the nature of sterility exhibited by irradiated M_1 plants, Ekberg (1969) analyzed 95 lines of barley in which partial sterility had been induced by various mutagenic agents. She found that in radiation treated material translocations and inversions were the most predominate form of chromosomal aberrations (82%), while these types

were in minority (27%) after EMS treatment. The majority in the latter case were lethals which cause abortion of seeds and/or gametes in heterozygous plants.

(2) A tendency exists for chemical-induced injuries to genetic material to remain latent over a period that may extend over many cell cycles, i.e. delayed effect. Since the formation of a chromosome rearrangement requires the simultaneous break of two chromosomes within the same cell, a potential rearrangement is lost if the two breaks open at different stages of the cell cycle. This could be due to a limited injury to DNA after the completion of its synthesis, resulting in chromatid type aberrations.

(3) The occurrence of a nonrandom distribution of chromosome breakages after treatment with chemical mutagens. In contrast to radiation, chromosome breaks induced by chemical mutagens are, in most cases, localized at certain regions known to be heterochromatic (Kihlman, 1963; Caspersson et al. 1969; Natarajan et al. 1969). Treatments of Vicia faba roots with 8-ethoxycaffiene and maleic hydrozide induced chromosome aberrations localized to the secondary constriction and the centromere region of metacentric M-chromosome, respectively (Kihlman, 1963). Caspersson et al. (1967) reported that chromosome breaks induced by quinacrine mustard occurred predominantly in the same well defined regions known in Vicia to be heterochromatic. In barley, Singh et al. (1970) found that about 50% of chromosomal breaks induced by L.S.D. were confined to the centromere region. The probable limitation of chromosome

aberrations to heterochromatin indicate that though the effect of chemicals may be due to alkylation of DNA, not all chromosomal DNA is susceptible (Grant and Heslot, 1966).

It should be kept in mind that localization of chromosome breaks at certain loci is a specific feature for any species treated with certain mutagen. Thus, generalization in only the broadest terms can be made.

MATERIAL AND METHODS

Two trisomic series of barley (H. vulgare L. 2n=15), one a 6-rowed type (OAC-21 x Montcalm) initially produced by Larter (personal communication) and the other a 2-rowed type cv. Betzes, Eslick and Ramage (1969) were used in the present study. Either dry seeds or trisomic seedlings and plants of each line were subjected to various mutagenic agents with the objective of producing individual telocentrics for the extra chromosome. Four experiments were carried out, each of which is discussed individually in the section to follow.

Experiment A

In this experiment, dry seeds of two trisomic stocks (Trisomics 4 and 5) were treated independently with two chemical mutagens, diethyl sulfate (DES) and ethylmethanesulfonate (EMS). Prior to treatment, seeds were presoaked in distilled water for 2 hours and subsequently transferred to freshly prepared 0.2M solutions of the mutagen. Treatment times were 2 and 24 hours at room temperature for DES and EMS respectively. Following treatment, seeds were washed thoroughly in tap water, chilled for one week ($+2^{\circ}C$) and then allowed to germinate on moist blotters. Trisomic plants were identified on the basis of root-tip analyses and were grown to maturity.

Experiments B and C

These experiments were designed to test the effect of hydroxylamine (HA) and γ-rays on meiosis. In experiment B, aqueous solutions of HA

were injected into the upper culm of individual tillers of trisomics 3 and 7 at the premeiotic stage (Sinha, 1967). The treatment was timed so that meiotic cells would be in contact with the solution during their actively dividing state. A concentration of 10 ug/ml of freshly prepared HA solution was used to which 5 ml/100 ml of solution of 0.01% "Tween 20" was added as a wetting agent. For control purposes, a few tillers were injected with distilled water containing "Tween 20" only. The treated heads and the control were tagged, and to prevent outcrossing each spike was bagged at the time of heading. In experiment C, five plants (cv. Betzes) trisomic for each of chromosomes 4, 5 and 6 were irradiated using a Co^{60} source. Two disomic plants served as control, one being cv. Betzes, the other OAC-21. Plants were treated when it was estimated that most of their tillers were initiating meiosis. A total of approximately 10,000 rads were given at a dose rate of 108 rads/hour. Following irradiation, both trisomic and control plants were allowed to reach maturity in the greenhouse.

Experiment D

In this experiment, the effect of low dosages of γ -rays alone and in combination with 5-fluorodeoxyuridine (FUdR) were applied to seedlings trisomic for chromosomes 1, 4 and 7, respectively. For each trisomic, seedlings were divided into three lots of 10 plants each and subjected to γ -irradiation from a Co^{60}-source adjusted to deliver 25 rads/second. The following treatments were applied:

Lot (1) 150 rads (6 sec.)

Lot (2) 500 rads (20 sec.)

Lot (3) 150 rads to seedlings that were pre-soaked in a 10^{-7}M aqueous solution of FUdR for three hours, then washed in tap water for one hour immediately prior to irradiation. Following treatment, the seedlings were planted.

All plant material, both treated and control was grown under a controlled temperature of 18 ± 2^{o}C with a 16 hour photoperiod. On the assumption that induced telotrisomics would exhibit higher fertility than their parental trisomics, those spikes of treated plants which exceeded the trisomic in seed-set were threshed individually, the remaining spikes were bulk harvested. Ten seedlings from each individual spike, as well as a random sample from the bulk, were cytologically analyzed on the basis of root-tip counts. If trisomic segregation occurred in individual samples, the data were pooled for statistical analysis. For Chi-square analyses of data on chromosome frequencies, the transmission rates of control for each chromosome were used to calculate the expected frequencies. Because of the low frequencies of telotrisomics in the control material, an average rate established over all trisomic lines was used to calculate the expected values.

RESULTS AND DISCUSSION

Experiment A

A comparison between the effect of the two chemical mutagens DES and EMS on dry seeds of trisomics 4 and 5, showed that DES caused 52 and 65% lethality respectively compared to 34 and 30% induced by EMS (Table I-1). This lethality may be due in part to the post-treatment storage period. Mikaelsen et al. (1968) found that storing barley seeds treated with DES and EMS increased the frequencies of fragments per cell and after 15 days of storage, the treated seeds failed to germinate. It seemed that trisomic seeds were more sensitive to both mutagens than disomics as revealed by a significant reduction in the frequencies of trisomics in treated populations ($P < .005$ for each trisomic). This could be expected since trisomic seeds are smaller than disomic ones (Tsuchiya, 1960) and small seeds show more physiological damage from mutagenic treatment than do large seeds (Heiner, 1963).

DES treatment resulted in a marked reduction in seed-set (Table I-1) accompanied by an increase in transmission of the extra chromosome (Table I-2). In contrast, EMS treatment did not result in the same degree of reduction in seed-set as did DES. However, it resulted in a slight decrease in transmission of trisomic-4 only. These results agree with the finding that DES tends to induce more acentric fragments (deficiencies) than does EMS (Mikaelsen et al., 1968), which could be compensated for by the presence of an extra chromosome, there-

Table I-1. Survival of Disomics and Trisomics Among M_1-Seedlings from Dormant Seeds Treated With EMS and DES.

Mutagen	No. of treated seeds	Trisomic 4					Trisomic 5				
		Survival %	Chr. number 14 %	Chr. number 15 %	X^2 (1)	Fertility %	Survival %	Chr. number 14 %	Chr. number 15 %	X^2	Fertility %
EMS	150	66.0	95.0	5.0	33.0**	41.2	70.0	83.0	17.0	5.8**	49.3
DES	100	48.0	96.0	4.0	18.0**	28.6	35.0	91.0	9.0	60.2**	35.9
Control (2)	120	95.3	66.0	34.0	-	61.4	93.1	72.0	28.0	-	63.5

(1) X^2 calculated for transmission of the extra chromosome.

(2) Soaked in distilled water.

Table I-2. Chromosome Segregation Among M_2-Seedlings from M_1-Irradiated Trisomics.

Mutagen	Trisomic 4					Trisomic 5				
	No. of Seedlings	Chr. Constitution 14 %	15 %	14+t (1) %	X^2 (2)	No. of Seedlings	Chr. Constitution 14 %	15 %	14+t %	X^2
EMS	180	78.9	21.1	-	9.6**	100	74.0	26.0	-	0.1
DES	124	56.5	43.5	-	6.3**	109	64.3	35.7	-	4.3*
Control (3)	136	67.3	32.2	-		111	71.0	28.1	0.9	

(1) 14+t: 2n + an extra fragment.

(2) X^2 calculated for transmission of the extra chromosome.

(3) Untreated trisomics.

fore favored higher frequencies of trisomics. Results from this experiment did not reveal any telocentrics.

Experiments B and C

Treatment of hydroxylamine and γ-rays at meiosis drastically decreased the size of population of the treated material so that conclusive results were not achieved. It was evident however, that injection of HA into the individual tillers (average of 3 tillers/plant) caused complete sterility of 15 plants of trisomic-3 and a reduced seed-set on 13 plants of trisomic-7 from which 67 seeds were recovered. Of these, 10 seeds (14.9%) germinated giving rise to disomics and trisomics only. Control plants (injected with distilled water and wetting agent) gave slightly lower seed-set than non-treated material (Table I-3).

On the other hand, irradiation with γ-rays during meiosis (Experiment C) severely damaged the plants and all tillers on trisomic plants were killed. Disomics, in contrast, matured and produced near-normal seed-set. Seeds harvested on 3 trisomic-6 plants (secondary growth) showed a parental chromosomal segregation with a decrease in the frequency of trisomics than untreated material (Table I-3).

It seems that some tillers of the control plants were more developed and probably had passed the stage of meiosis before treatment. Hence, radiosensitivity of disomics and trisomics could be attributed to differences in ontogenetic stages of plants at the time of treatment. In general, results from treatments at meiosis were inadequate and did not

Table I-3. The Effect of HA and γ-Rays on Trisomic and Disomic Plants Treated During Meiosis.

Treatment	Trisomic Line	No. of Treated Heads	No. of Seeds	Germination %	Chr. Segregation 14 %	15 %	14+t %	No. of Seedlings Examined
HA (injection)	3	43	0	-	-	-	-	-
	Control	14	79	85.1				
	7	35	67	14.9	80.0	20.0	-	10
	Control	11	187	82.3				
γ-rays (10 Kr)	4	-	-	-				-
	5	-	-	-				-
	6		92 (1)	90	87.8	12.2	-	81
	Control: 6 (2)	-	266	93.6	72.2	27.8		249
	OAC-21	4 (3)	192	94.0	-	-		-
	Betzes	3 (3)	84	86.0	-	-		-

(1) Secondary growth.

(2) Untreated material.

(3) One disomic plant each.