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Colchicine Induced Heritable Modifications in Leaf Mesophyll Cells of Wheat, (*Triticum aestivum* L.)

[1]L. Hassan, [2]S.D. Ahmad, [3]A. Okumus and [3]M. Wazuddin
Department of Genetics and Plant Breeding,
Bangladesh Agricultural University, Mymensingh 2202, Bangladesh
[1]Institute für Pflanzenbau and Pflanzenzüchtung,
Georg-August-Universitat, Von-Siebold Str. 8, D-37075 Göttingen, Germany
[2]University College of Agriculture Rawalakot, Poonch, Azad Kashmir, Pakistan
[3]Ondokuz Mayis University, Agriculture Faculty, Biometry-Genetics Department, Samsun, Turkey

Abstract: A 3 hr. period treatment with a 0.2% aqueous solution of colchicine was given to one week old seedlings of seven inbred lines of wheat (*Triticum aestivum* L.) plants. Comparisons were made in the CT1 generation between the untreated controls (2x) and colchicine treated plants (C2x) for variation of leaf mesophyll cell size and chloroplast number. Differences were 4ound in that the cell section area and the number of chloroplasts per cell in all the seven lines investigated were significantly greater in the C2x selection compared with the 2x controls using coded determination. Heritable differences were found such that the cell selection areas and the numbers of chloroplasts per cell were significantly greater in the C2x treatment in all the lines. In each case there was an enhancement of chloroplast number independent of the variation in cell size.

Key words: *Triticum aestivum*-cell size-chloroplast number-colchicine-induced changes

Introduction

There have been experiments performed in which colchicine has been used as an agent to investigate the mechanism and time of meiotic chromosome pairing. Colchicine treatment applied to seedlings of diploid rice plants induces mixoploidy. When diploid parts of the treated plants (C2x) are separated out and compared as plants with untreated controls (2x) there are marked effects on the development of inbred lines due to the colchicine treatment. These effects have been studied for a number of agronomic characters, including tiller number and fresh weight of leaves and have been found to persist over several years during which lines of inbred rice were kept (Hassan *et al.,* 1993a). Similar types of induced changes in ryegrass are heritable and are also observed in *the* CT1 plants grown from seed produced by selfing the CTO (Francis and Jones, 1989). Confirmation of these induced heritable changes has been obtained in another series of experiments using CT1 plants from lines of both *L. perenne* and *L. multiflorum* (Hassan *et al.,* 1989). The main findings of the agronomic characters were that colchicine induced an increase in tiller number and fresh weight of leaves, of the order of 25% averaged over several lines, and that the changes were directional in the lines involved.

In a later study of Francis and Jones, 1990 with CT1 lines of *L. perenne*, observations were made on cell characters, viz. leaf mesopyll cell size and chloroplast number per cell. Heritable differences were again found, such that cell size was less in the C2x compared with the 2x in four out of five lines, and chloroplast number were also lower in two out of the five lines. in one line however, there was a higher number of chloroplast per cell in the C2x compared with its 2x control. This publication presents new results for colchicine-induced heritable variation in leaf mesophyll cell size and chloroplast content in seven inbred lines of wheat plants. In all the lines significant increase in cell plant area and chloroplast number were observed.

Materials and Methods

The materials and the methods for treating the seedlings and growing the plants to produce the CT1 generation are identical to those described in two previous publications on wheat and ryegrass (Hassan *et al.,* 1993a). The same wheat varieties were used for the present work as were used in previously reported studies on "agronomic" character variation (Hassan *et al.,* 1993b). As explained in the previous publications, CTO refers to the treatment generation in which the colchicine was applied to the seedlings and CT1 is the generation following that in which the treatment was given and which was obtained by selfing the CTO plants. The terms 2x and C2x refers to the control and to the colchicine treated "isogenic" lines respectively.

Leaf material was taken in March 1999, four months after germination. In each line five control (2x) and five treatment (C2x) plants from the CT1 generation were earmarked at random for the experiment. In order to sample leaves of corresponding physiological age, in the different lines, the material of wheat plants was taken from third leaf of a tiller in which the fourth leaf was half emerged. Five 1 mm transverse section were cut from a point 3 cm below the leaf tip. The samples were fixed in 3.5% (w/v) gluteraldehyde in small stoppered bottles, and the bottles were then placed on a rotor in the dark at 4°C for 1 hour. Following gluteraldehyde treatment the segments were transferred to 0.1 M sodium ethylenediaminetriacetate (Na EDTA) at pH 9 and shaken in a waterbath at 60°C for 3 hours. After cooling the samples were stored in 70% alcohol at 4°C until required (Pyke and Leech, 1987).

One piece of leaf tissue from a plant was macerated on a slide in a drop of 0.1 M NaEDTA and viewed under Normarski differential interference optics. Individual cells, or small groups of cells, which showed a clear appearance were selected and photographed in three planes of focus to allow all the chloroplasts of the cells to be counted. After processing the

films, the number of chloroplasts per cell was determined using the three photographs of each cell and marking off the chloroplasts individually. Eleven cells were recorded in this way from each of the five plants in each line, for both the 2x and C2x treatments.

Cell plan areas were determined using a computer-linked digitizing system. The outline of each cell was traced from each of the three photographs, and a mean value for cell plan area calculated. The values as μm^2, were determined using a conversion factor based on the magnification of the cells on the photographs.

Results

The results for mean cell plan areas and mean chloroplast number per cell in the 2x and C2x treatments, for the seven lines, *are* given in Table 1. Mean value and standard deviations per inbred line, together with probabilities from t-test to compare treatment effects within lines, appear in Table 2. There are significantly highevalues for both characters in the C2x treatment compared with the 2x treatment in all the lines. For cell plan area the C2x value is 162 and 150% that of the 2x in lines Anza and Soalike respectively. The corresponding increase for chloroplast numbers in these lines are 154 and 142% .

There is also genotypic variation between lines for both cell plan area and chloroplast number per cell in the C2x material, but not in the 2x controls. The variation in the C2x is highly significant, and the extremes of values for cell plan area are from 701.7 μm^2 in Ad-66.1 to 957.7 pm^2 in Icanchon; and for chloroplast per cell the corresponding figures are 23.9 and 35.9 in the same two lines. It appears that the response to colchicine has some interaction with genotype.

Variation between and within lines was also examined to see the variance differed for the 2x and C2x treatment. The variance ratio was obtained by dividing the larger of the two variances (2x or C2x) by the smaller. The only significant comparison is between lines cell plan area, where the C2x has a greater variance than the 2x.

Table 1: Mean cell areas (μm^2) and mean number of chloroplasts per cell in 2x and C2x plants of seven inbred lines of wheat plants, in the Ct1 generation. Twelve cells were analysed in each plant

Line	2x		C2x	
	Cell plan area	Chloroplasts per cell	Cell plan area	Chloroplasts per cell
Ad-66-1	435.9	19.7	652.0	25.8
n = 5	476.0	19.6	783.9	22.0
	564.8	18.0	524.9	21.9
	665.9	17.5	693.1	26.8
	498.0	20.3	854.7	22.8
Aghrani	543.9	20.9	888.6	23.8
n = 5	5017	19.2	902.3	27.9
	567.9	19.4	692.0	24.4
	599.8	17.3	871.0	26.9
	601.8	18.8	764.1	28.0
Anza	398.9	23.9	683.0	30.6
n = 5	376.1	20.6	601.8	29.4
	462.8	22.4	886.0	35.1
	483.2	23.1	723.9	39.6
	523.1	24.9	767.8	33.4
DSN-61	664.8	23.7	990.8	35.7
n = 5	571.8	19.8	823.6	30.9
	501.2	21.7	856.7	30.7
	676.7	22.0	721.9	23.1
	552.0	22.8	657.9	27.8
Kanchon	701.7	29.9	1098.9	40.8
n = 5	754.9	26.1	952.8	42.8
	608.8	27.6	1108.9	31.3
	675.9	25.9	795.0	34.5
	742.9	28.8	843.0	29.8
Sonalika	621.9	26.6	899.6	42.6
n = 5	598.6	25.9	951.9	40.1
	622.3	23.8	1034.9	35.8
	678.0	25.7	756.1	32.0
	532.9	24.7	942.9	28.5
YC-17	777.7	22.9	803.9	25.8
n = 5	643.8	19.6	821.4	30.5
	610.7	20.3	674.9	29.1
	766.0	21.5	1108.0	34.9
	569.5	22.5	978.1	37.7

Discussion

Colchicine treatment applied to seedlings has now been shown to affect a wide range of quantitative characters in both perennial and Italian ryegrass (Francis *et al.,* 1990), in rice plants (Hassan *et al.* 1993a) and in wheat (Hassan *et al.,* 1993b). The characters concerned included tiller number, fresh and dry weight of leaf material, length of head, spikelets per ear and flowering time; to which he may now add the cell characters of cell plan area and chloroplast number per cell. In general the effect of the treatment are positive and directional such that the colchicine produces more, and more quickly as judged by heading date. The exceptions to this generalisation are one line of *L.muftiflorum* where the C2x had significantly fewer tillers than the 2x (Hassan *at al.* 1989) and cell size and chloroplast number in some lines of *L. perenne* where the C2x have lower values than the 2x (Francis *et al.,* 1990). At the cell level in particular it seems that heritable variation can be produced to deviate from that of the control lines in either direction. In this respect our results parallel those reported in cotton (Luckett, 1989). Luckett treated seedlings of a commercial cultivar of *Gossypium hirsutum* with various concentrations of colchicine at the seedling stage and then studied the C2 and C3 generations in field experiments for morphological variants and for variation in quantitative characters. As in ryegrass no morphological variants were found, but there was considerable quantitative heritable change for several characters, and in this case no cytological observations were made, but presumably the C2 and C3 generations produced by self ing remained at their normal tetraploid level.

In this paper, as in the previous study of cell characters in ryegrasses (Francis *et al*., 1990) and rice plants (Hassan and Wazuddin, 2000), it is interesting to note not only the potential for inducing heritable variation, but also the way in which cell size and chloroplast number can be changed independently. The general positive correlation between chloroplast numbers and cell size holds for all lines and both treatments when considered separately: The differences are between lines and between treatments within lines. A real possibility now exists, therefore, for the experimental manipulation of these cell characters within inbred lines of ryegrasses, rice, wheat and may be within other species as well. The limits to this induced variation are unknown and await a clear definition of the treatment conditions. To date all of our experiments have utilised the standard treatment on one week old seedlings of 0.2% colchicine for 3 hour at room temperature. We are investigating modifications of treatment

Table 2: Mean and standard deviation per inbred line for cell section areas and chloroplast per cell in the 2x and C2x treatments of the C1 generation of seven inbred lines, together with probabilities based on t-tests for the comparison of treatments within lines using individual cell data In = 60, 5 plants per line, 12 cells per plant)

	Cell section area (μm^2)			Chloroplast per cell		
	2x	C2x	t	2x	C2x	t
Ad-66-1	528.1 ± 90.1	701.7 ± 126.5	*	19.0 ± 2.3	23.9 ± 2.3	***
Aghrani	563.0 ± 41.9	823.6 ± 91.6	***	19.1 ± 1.3	26.2 + 2.0	***
Anza	448.8 ± 60.6	728.5 ± 108.1	***	23.0 ± 1.6	33.6 ± 4.0	***
DSN-61	593.3 + 75.4	810.2 ± 128.4	*	22.0 ± 1.5	29.6 ± 4.6	**
Kanchon	696.8 ± 58.5	959.7 ± 143.6	**	27.7 ± 1.7	35.9 ± 5.7	*
Sonalika	610.7 ± 52.4	917.1 ± 102.4	***	25.3 ± 1.1	35.8 + 5.8	***
YC-17	673.5 ± 03.6	879.1 ± 169.4	*	21.4 ± 1.4	31.6 ± 4.7	***
Mean	587.7	831.5		22.5	30.9	
LSD%	90.5	164.1		1.8	5.7	

*, **, *** Significant at p = 0.05, p = 0.01 and p = 0.001, respectively

which involve concentration of colchicine, duration and temperature of treatment and applications at different stages of seedling development. Studies are also in hand to test for similar effects in other crop plant species.

The mechanisms underlying these induced changes in plant growth and development remain speculative, but reorganisation of the nuclear and chloroplast genomes as well as alterations of the cytoskeleton are lines of inquiry which could profitably be persuaded.

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