

DIFFERENTIAL EFFECT OF SOIL ACIDITY AND LIME TREATMENT ON THE CHROMOSOMES OF TWO WHEAT CULTIVARS*

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ABSTRACT

The meiotic behaviour of pure lines of two wheat (*Triticum aestivum* (L.) Thell.) cultivars was studied in a growth chamber experiment, using four levels of lime supplements. Both cultivars (PAT 7392 and CNT 10) are aluminum tolerant and were used due to contrasting meiotic indices (80 and 98 respectively). The frequencies of several chromosomal abnormalities were recorded for each cultivar at each level of lime addition and analyzed using χ^2 tests for linearity and regression. Chromosome stickiness, breaks, early bivalent separation, univalents and precocious division of univalent centromeres showed a linear dependence on soil correction, while nonoriented bivalents, unequal segregation, micronuclei, laggards and bridges showed a non-linear relationship or absence of dependence. A clear differential effect of the lime treatment was observed in the two cultivars, CNT 10 being insensitive to it, while in PAT 7392 significant decreases in the frequencies of stickiness, univalents and chromosome breaks occurred. Low soil pH may be at least partially responsible for the high rates of chromosome abnormalities found in wheat grown in southern Brazil.

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INTRODUCTION

High frequencies of chromosomal abnormalities were observed in mitotic and meiotic divisions in wheat plants cultivated in the southern region of Brazil. Somatic instability with dicentric chromosomes, rings, bridges, micronuclei and numerical mosaicism were observed in root tip mitosis (Guerra Filho and Moraes Fernandes, 1977). In pollen mother cells (PMCs), pairing disturbances causing high rates of univalents, chromosomal breaks, bridges by non-terminalization of chiasmata in anaphase I (AI) and telophase I (TI), stickiness, laggards and micronuclei in pollen quartets were found in very high frequencies. Studies have demonstrated that various environmental components, as well as the genotype, are involved in the high rates of chromosomal abnormalities observed in these strains of wheat (Bodanese Zanettini *et al.*, 1979; Del Duca and Moraes Fernandes, 1980; Sereno *et al.*, 1981; Moraes Fernandes, 1982; Bodanese Zanettini *et al.*, 1983; Moraes Fernandes *et al.*, 1985) and in triticale (Falcão *et al.*, 1981).

Germ plasm carrying the dwarf genes from the Japanese variety Norin 10, introduced from CIMMYT (Centro Internacional de Mejoramiento de Maiz y Trigo) had the highest levels of meiotic instability observed in cultivars of different origins (Moraes-Fernandes *et al.*, 1985). Del Duca (1983, unpublished) evaluated the genealogy of 117 wheat cultivars that had variable Meiotic Indexes when cytologically analyzed at CNPT (Centro Nacional de Pesquisa de Trigo), EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) and concluded that most of the meiotically unstable cultivars had Norin 10 genetic material in their pedigree. Love's Meiotic Index (1949) is the percentage of normal pollen quartets.

The soils of the wheat growing areas in southern Brazil are usually acidic and have a low content of available phosphorous and organic matter. Soil pH by itself does not directly affect the plant growth development, except for values below 4.2. However, the solubility of various essential and nonessential elements is affected by the low pH in acid soils.

Aluminum (Al) and manganese (Mn) are the main causes of impaired plant growth when they became too soluble in acid soils (Malavolta *et al.*, 1976; Foy *et al.*, 1978). The initial site of Al injury occurs in the root system, but the foliar symptoms are not always easily identified (Foy, 1974). An excess of Mn, however, generally affects plant tops more severely than roots and produces more definitive foliar symptoms (Foy *et al.*, 1978). Hence, in acid soils containing high levels of Al and Mn the plant growth reduction observed may be wrongly attributed to Mn toxicity when Al toxicity is the most important of the two factors (Foy *et al.*, 1978). In addition, Siqueira (1980) studied wheat and soybean (*Glycine max* Merr.) grown on acid soils in Southern Brazil, and reported that there was a clearer relationship between the excess of aluminum than of manganese in problems of plant growth and of developmental stability.

Al has been shown to interfere with cell division in plant roots, as well as with the uptake, transport and utilization of Ca, Mg, P, K and water, and with certain enzymes activity (Foy *et al.*, 1978). Clarkson (1965) found that Aluminum treatment caused a cessation of onion (*Allium cepa* L.) elongation which was correlated with the disappearance of mitotic figures in the root meristems.

Al was localized in the epidermis and regions where cells actively divided, and accumulated mainly in the nuclei and cell walls (Matsumoto *et al.*, 1976). Morimura and Matsumoto (1978) showed a clear binding of Al to DNA-phosphate.

This same element has been reported to decrease both the rate of DNA synthesis (Sirover and Loeb, 1976; Wallace and Anderson, 1984), and DNA template activity (Morimura and Matsumoto, 1978; Matsumoto and Morimura, 1980).

Studies on the influence of metal ions in *Allium* root cells revealed that Al, even in low concentrations, induced certain general effects on the chromosomes, mainly manifested as stickiness. However, the disintegration of nucleoli and their extrusion from the nucleus into the cytoplasm was the most striking response to toxic treatments with Al (Levan, 1945; Fiskesjö, 1983a,b).

Zanella *et al.* (1984) demonstrated that the PMCs of Cotiporã wheat cultivar plants grown on acid soils (2.9 ppm Al) showed increased numbers of univalents and micronuclei, and increased stickiness of chromosomes of PMCs, when compared to the PMCs of plants of the same cultivar grown on soils with the pH corrected by lime. The observations of these authors stimulated the present investigation. The objectives of our study were to determine the effects of soil pH correction by lime on chromosomal instability and to develop a better understanding of genotype-environment interaction.

MATERIAL AND METHODS

Two spring wheat cultivars (PAT 7392 and CNT 10) with the same growth cycle were used for this study. PAT 7392, which has Brazilian and Mexican parentage, is tolerant to aluminum, and is known to have very high levels of meiotic instability. CNT 10, which has no Mexican parentage, is also tolerant to aluminum but is meiotically stable. The plants analyzed were from pure lines obtained from one plant of each cultivar previously analyzed for Meiotic Index (MI). The MI was 98 for CNT 10 and 80 for PAT 7392.

A Dark Red Dystrophic Latossol soil originated from basalt, very acidic with a high soluble aluminum content, low phosphorous availability and medium to low organic matter content was used. Lime was applied to the soil at rates of 0, 1/4, 1/2, and 1 times the amount required to produce pH 6. The lime requirement was 10.6 t/ha, and lime treatments were applied as a mixture of CaCO₃ and MgCO₂ in a 3:1 ratio. The soils were incubated for four months, until their pH reached equilibrium (Table I).

Table I - Amounts of lime added, pH values, and available Al concentration in the soils tested.

Lime (t/ha)	pH	Al (me/100 g soil)
0.00	4.0	3.3
2.65	4.6	1.5
5.30	5.0	0.4
10.60	5.8	0.0

The seeds were sown in pots that were kept in a green-house until just after seedling emergence. Seedlings were transferred to a growth chamber ("CONVIRON", mod. PGW 36) with 12 hours of light and 12 hours of darkness, at a temperature of 20°C during the day and 14°C at night, and 80% humidity.

The spikes at anthesis were fixed in Newcomer (1953) solution and the PMCs stained with propionic carmin.

Associations between the frequencies of different kinds of chromosome irregularities and lime amounts were determined by the usual χ^2 with three degrees of freedom. This overall χ^2 was then partitioned into a χ^2 for linearity and one due to the regression of the aberration frequencies on the lime amounts identified (Fleiss, 1981). The hypothesized linear relationship was tested with two degrees of freedom. When this hypothesis was accepted (χ^2 for linearity nonsignificant), the linear regression coefficient (b) was analyzed with one degree of freedom (χ^2 of regression). A statistical significance of the χ^2 for linearity would indicate that the relationship between the variables was not linear, precluding an estimate of a linear slope.

RESULTS

The different kinds of irregularities found in both CNT 10 and PAT 7392 were described in previous reports (Bodanese-Zanettini *et al.*, 1979, 1983; Moraes Fernandes *et al.*, 1985). A total of 3,334 PMCs in metaphase I plus anaphase I, and 7,205 pollen quartets from spikes of 77 plants (40 CNT 10 and 37 PAT 7392) were analyzed, and the frequencies of the abnormalities observed in each cultivar (Table II).

Table III includes the statistical analysis of the relationship between chromosome irregularities and amounts of lime added to the soil. In both cultivars the frequencies of PMCs with nonoriented bivalents, unequal segregation of chromosomes and pollen quartets with micronuclei depended on the lime amounts; the relationship, however, was not linear (χ^2 for linearity varying from 7.03 to 60.70, all statistically significant). For nonoriented bivalents, CNT 10 had higher frequencies than PAT 7392 (Table II). How-

ever, PAT 7392 had higher frequencies of cells with unequal segregation and micronuclei. There was no agreement between frequencies of abnormalities and dependence on soil correction, in CNT 10 and PAT 7392.

Table II - Percentages of pollen mother cells in metaphase I, anaphase I and pollen quartets with the indicated chromosome aberrations in plants of CNT 10 and PAT 7392, at four levels of lime added to soil.

Chromosome aberrations	CNT 10				PAT 7392			
	Levels of lime (t/ha)							
	0.00	2.65	5.30	10.60	0.00	2.65	5.30	10.60
No. of cells at MI	221	277	375	235	100	207	296	195
Nonoriented bivalents	11	11	3	6	6	1	3	6
Stickiness	92	96	93	94	85	85	72	52
Breaks	9	12	11	7	13	14	8	7
Early separation	5	4	3	6	30	30	27	17
Univalents	1	3	2	1	40	28	22	16
No. of cells at AI	212	154	265	236	57	115	234	155
Unequal segregation	3	8	1	1	28	11	13	14
Laggards	2	1	1	1	15	15	12	9
Bridges	14	20	14	13	7	7	8	9
Stickiness	83	83	84	88	75	77	63	39
Breaks	4	4	2	3	12	10	5	3
Precocious division of univalent centromeres	1	1	1	2	15	15	8	4
No. of quartets	900	1000	1000	1100	118	887	900	1300
Micronuclei	8	3	6	10	36	41	21	23

No other relationships between chromosome irregularities and lime amounts were observed in CNT 10 (χ^2 : 1.02-4.39, all nonsignificant). This cultivar maintained a stable response to different soil corrections (Table III, Figure 1A-E). PAT 7392 had a different behaviour. The frequencies of cells with breaks in metaphase I, laggards (Figure 1E) and bridges (Figure 1D) in anaphase I were independent of the soil treatments (χ^2 :

Table III - Abnormalities observed in PMCs of the cultivars CNT 10 and PAT 7392, at four levels of lime: values obtained for χ^2 overall (χ^2) (GL = 3), χ^2 linearity (χ^2_{lin}) (GL = 2), χ^2 regression (χ^2_{reg}) (GL = 1) and slope (b).

	CNT 10				PAT 7392			
	χ^2	χ^2_{lin}	χ^2_{reg}	b	χ^2	χ^2_{lin}	χ^2_{reg}	b
Metaphase I								
Nonoriented bivalents	20.83***	13.23**	-	-	9.64*	7.03*	-	-
Stickiness	4.16	3.90	0.05	1	65.37***	3.10	61.60***	-38
Breaks	4.39	2.94	1.28	-3	7.58	2.66	5.48	-8
Early separation	3.63	3.28	0.38	1	11.07*	0.97	9.94**	-15
Univalents	4.21	3.67	0.35	-1	23.32***	2.13	19.78***	-21
Anaphase I								
Unequal segregation	21.13***	16.23***	-	-	9.99*	8.59*	-	-
Laggards	1.02	0.58	0.67	-1	2.88	0.10	2.14	-6
Bridges	4.18	3.57	0.25	-2	0.47	0.03	0.43	2
Stickiness	3.08	0.26	2.53	5	50.34***	2.84	45.78***	-43
Breaks	1.99	1.53	0.58	1	9.63*	1.35	7.94**	-9
Precocious division of univalent centromeres	1.37	0.33	1.57	1	12.98**	1.55	10.97***	-12
Pollen Quartets								
micronuclei	22.82***	14.57***	-	-	116.41***	60.70***	-	-

*P < 0.05, **P < 0.01, ***P < 0.001.

7.58, 2.88 and 0.47, all statistically nonsignificant; Table III). But there was a significant dependence of the frequencies of cells with stickiness (Figure 1A,B), early bivalent separation, univalents (Figure 1C), breaks in anaphase I, and precocious division of univalent centromeres, on the lime doses. The frequencies of such abnormalities decreased linearly (all χ^2 for deviations of linearity were nonsignificant) with increasing amounts of lime (all χ^2 for regression were significant at least at the 0.01 level) (Table III). The highest reduction (as ascertained from b , the slope of the regression) occurred in frequencies of cells with stickiness: 38% in metaphase I and 43% in anaphase I in plants grown in soils to which the amount of lime required to produce pH 6 (10.6 t/ha) was added. This suggests an expected reduction of 3.8% of cells with stickiness in metaphase I for each 10% (1.06 t/ha) of the total amount of lime applied to the soil. In the other abnormalities, this reduction varied from 9% (breaks) to 21% (univalents) for soils totally corrected.

DISCUSSION

The influence of environmental factors on the incidence of chromosomal abnormalities is known from studies that have focused primarily on temperature effects (Pao and Li, 1948; Rana, 1965; Utkhede and Jain, 1974; Al'Tergot *et al.*, 1978; Bodanese-Zanettini *et al.*, 1979, 1983). Genotypic variables, however, seem to be responsible for intervarietal differences in the frequencies of abnormalities in chromosomal behaviour at mitosis or meiosis (Hollingshead, 1932; Riley and Kimber, 1961; Guerra-Filho and Moraes-Fernandes, 1977; Moraes-Fernandes, 1982; Bodanese-Zanettini *et al.*, 1983).

There is evidence that the genotype plays a prominent role in the occurrence of a certain type of chromosomal bridge in anaphase I and telophase I that is caused by a delay in chiasmata terminalization. Significant differences among cultivars were found in frequencies of cells with these bridges and there was a significant correlation ($r = 0.66$; $P < 0.01$) between the mean frequencies of PMCs with bridges in mother plants and their progeny (Bodanese-Zanettini *et al.*, 1983). According to Zanella *et al.* (1984) the average frequencies of PMCs showing bridges in the cultivar Cotiporã did not differ between samples grown in soil with uncorrected pH and samples grown in soil with corrected pH. In the present investigation PAT 7392 and CNT 10 also did not show differences in bridge frequencies when the levels of lime varied (Figure 1D), but CNT 10 had uniformly higher frequencies.

Lime application also did not modify the frequencies of PMCs with laggards (Figure 1E). But PAT 7392 had consistently higher frequencies, emphasizing again the different behaviour of the genotypes.

Previous reports have shown the importance of the genotype in the occurrence of univalents in wheat cultivars (Hollingshead, 1932; Riley and Kimber, 1961; Watanabe,

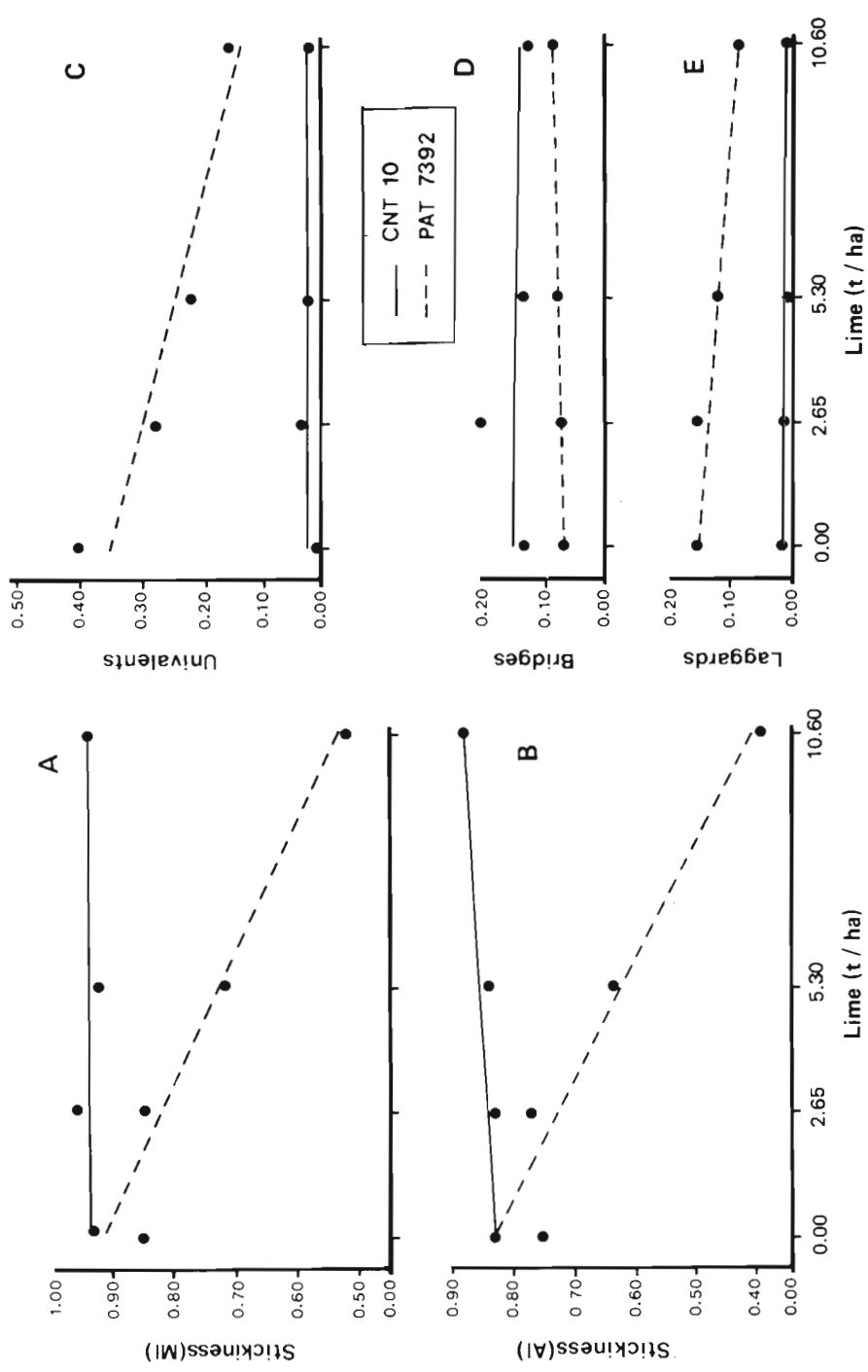


Figure 1 - Frequencies of PMCs that shoed stickiness in methaphase I (A), stickiness in anaphase I (B), univalents (C), bridges (D) and laggards (E) in CNT 10 and PAT 7392 plants at four levels of lime added to the soil.

1962; Utkhede and Jain, 1974). Previous studies also showed high parent-offspring correlations in the frequencies of cells with univalents. Thus, Moraes-Fernandes (1982) reported $r = 0.87$ ($P < 0.01$); and Bodanese-Zanettini *et al.* (1983), $r = 0.48$ (P near the 5% level of significance) in wheat, while Falcão *et al.* (1981) obtained $r = 0.86$ ($P < 0.05$) in Triticale.

Reports have attributed the occurrence of chromosomal breaks to deficiencies of magnesium (Steffensen, 1953) and calcium, potassium, and phosphorus (Das and Sen, 1976). Environmental pollution (Wuu and Grant, 1967; Amer and Farah, 1968, 1980; Reddy and Rao, 1969; Amer and Ali, 1974; Ravindran and Ravindran, 1978) and virus infections (Sandfaer, 1973) have also been reported to cause chromosome breaks. Other studies showed low parent-offspring correlations, indicating that the genotype may not have a significant effect on this type of chromosome abnormality (Falcão *et al.*, 1981; Moraes-Fernandes, 1982, and Bodanese-Zanettini *et al.*, 1983).

It has been well demonstrated (Levan, 1945; Fiskesjö, 1983b; Zanella *et al.*, 1984) that an excess of aluminum and/or manganese in soil are involved in the incidence of stickiness.

Our results show a genotype-environment interaction involving CNT 10 and PAT 7392 and different amounts of lime in the soil, influencing the occurrence of stickiness, univalents and chromosomal breaks. Low soil pH may be at least partially responsible for the high rates of chromosome abnormalities found in some wheat cultivars grown in southern Brazil.

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RESUMO

O comportamento meiótico de linhas puras de duas cultivares de trigo (*Triticum aestivum* (L.) Thell.) foi estudado num experimento, em câmara de crescimento, utilizando-se quatro níveis de calcário adicionado ao solo. Ambas as cultivares (PAT 7392 e CNT 10) são tolerantes ao alumínio e foram usadas devido a índices meióticos contrastantes (80 e 98, respectivamente). As frequências de várias anormalidades cromossômicas foram registradas para cada cultivar em cada nível de calcário e analisadas utilizando-se testes de χ^2 para linearidade e regressão. Aderências cromossômicas, quebras, separação antecipada de bivalentes, univalentes e divisão precoce dos centrômeros de univalentes mostraram uma dependência linear à correção do solo,

enquanto bivalentes não orientados, segregação desigual, micronúcleos, retardatários e pontes mostraram dependência não linear ou ausência de dependência. Observou-se um claro efeito diferencial do tratamento de calcário nas duas cultivares, CNT 10 sendo insensível ao mesmo, enquanto em PAT 7392 ocorreram decréscimos significantes nas freqüências de aderências, univalentes e quebras cromossômicas. O baixo pII do solo pode ser pelo menos parcialmente responsável pelas altas taxas de anormalidades cromossômicas encontradas no trigo cultivado no sul do Brasil.

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