

Plant height reduction and disease resistance in wheat (*Triticum aestivum* L.) cultivar IAC-18 by gamma radiation-induced mutations

Augusto Tulmann Neto¹, Carlos E.O. Camargo², Marcelo Correa Alves³,
Armando Pettinelli Junior² and Antonio W.P. Ferreira Filho²

ABSTRACT

Mutation induction by gamma rays was used to obtain plants with reduced plant height and increased disease resistance in wheat (*Triticum aestivum* L.) IAC-18 cultivar. Seeds were irradiated with 40 krad and selection began from the M2 generation. Nine selected lines were compared with the original IAC-18 cultivar for three years in seven advanced trials carried out in various locations, under and without irrigation. Four of the lines had a significant plant height reduction and a lower percentage of lodging, while grain yield and aluminum tolerance remained unaltered. All the selected lines were more resistant to the causal agents of leaf rust and three had immunity to stem rust. Among the resistant lines, some did not have reduced grain yield or tolerance to aluminum, maintaining the IAC-18 cultivar characteristics. This showed the usefulness of this method for correction of defects in cultivars which have desirable agronomic traits.

INTRODUCTION

The increase in the cultivated area and production of wheat in São Paulo has been due to the recent release of new cultivars adapted to the new wheat cultivation areas. Among the characteristics breeding programs have attempted to meet, the most important are semi-dwarf size, Al³⁺ toxicity tolerance and disease resistance (Camargo, 1993). Cultivar IAC-18 (BH 1146 x S-12), released by the Agronomic Institute at Campinas, in spite of its height had all these desirable characteristics at the time of its release (Camargo *et al.*, 1985), but later it was found to be susceptible to leaf and stalk rust.

Mutation induction has resulted in a large number of *Triticum* cultivars. About 97 mutants were released directly to producers and 27 included in breeding programs which produced mutant derived new cultivars (Micke *et al.*, 1990). These mutants resulted from seeds treated with different types of radiation (X and gamma rays) and the mutants were different from the original cultivars, in characteristics such as life cycle, height, disease resistance, adaptability, etc...

In Brazil, there are various examples of wheat mutants with height reduction (Veiga *et al.*, 1982a), resistance to stalk rust (Osorio, 1972; Veiga *et al.*, 1982b) and earliness (Philipovski *et al.*, 1984). In one of these cases, reported by Osorio (1972), a mutant was introduced into a breeding program which developed two derived cultivars. It became the first Brazilian example of development of a cultivar using mutation induction.

¹ Centro de Energia Nuclear na Agricultura, Universidade de São Paulo (USP), Caixa Postal 96, 13400-970 Piracicaba, SP, Brasil. Send correspondence to A.T.N.

² Instituto Agrônomo, Caixa Postal 28, 13001-970 Campinas, SP, Brasil.

³ Centro de Informática na Agricultura/USP, Caixa Postal 9, 13416-260 Piracicaba, SP, Brasil.

MATERIAL AND METHODS

Seeds of the wheat cultivar IAC-18 were treated with gamma rays in the ^{60}CO source at Centro de Energia Nuclear na Agricultura (CENA), Universidade de São Paulo (USP) in Piracicaba, SP.

Sensitivity determination for choice of the gamma ray dosage

Determination of sensitivity to gamma rays was carried out in 1984. Seeds with approximately 12% moisture content were irradiated with 10, 15, 20, 25, 30, 35, 40, 45 and 50 krad at a dosage rate of 129 krad/h. A randomized complete block design experiment with five replications and thirty seeds per plot was used. Seeds were sown in wooden boxes with soil and organic material in a greenhouse. Assessment was carried out 20 days after sowing, with records on seedling height and appearance being taken.

Obtaining the M_1 and M_2 generations and the beginning of selection

Based on the results of the experiment previously described, irradiation of 160,000 seeds with 40 krad was carried out to obtain the M_1 generation. Sowing took place in 1985 in the IAC Experimental Station field in Tatuí, SP. The M_1 generation was harvested by the bulk system, resulting in 1,075,000 M_2 seeds. A sample of 320,000 seeds was taken, corresponding to twice the number of irradiated seeds for the M_2 generation sowing. This was done to obtain in this sample at least one seed from each of the plants that made up the M_1 generation. The sowing was carried out in Tatuí, in May 1986. Five-meter rows spaced at 0.20 m were used. One row of the original IAC-18 was planted every ten rows as a control. Selection, specially of plants shorter than the control and resistant to stalk and leaf rust and helmintosporiosis, took place at the beginning of maturity. At complete maturity one ear from each selected plant was harvested.

Handling of the M_3 and M_4 generation

In May 1987 the M_3 seeds from plants selected in the previous cycle were sown on an ear-to-row basis. One-meter rows spaced at 0.20 m were used. One row of the original (control) cultivar was planted at every ten experimental rows. Selection between and within progenies for the mentioned traits was carried out at maturity. From every selected plant a single ear was harvested.

In May 1988 the M_4 seeds from each ear were again sown in an ear-to-row basis maintaining the same spacing and row length and arrangement as in the previous year. Selection of the best progenies was carried out at maturity. The selected material was harvested and the seeds were assessed in the preliminary yield trial the following year.

These M_4 seeds were tested in nutritive solution containing 3, 6 and 10 mg/l of Al^{3+} , using the method described by Moore *et al.* (1976) and Camargo and Oliveira (1981).

Preliminary yield trials

Seeds from the M_5 progenies derived from the irradiated populations were sown in two three-meter rows spaced at 0.40 m at the Tatuí Experimental Station in May 1989. The soil fertility was corrected with lime and fertilizer and spray irrigation was used when necessary.

At the beginning of maturity the progenies were assessed for height, cycle, disease resistance and grain yield. The best were selected for study in the advanced line trials.

Advanced yield trials

Nine mutant lines of IAC-18 obtained by irradiation treatment were selected and tested along with the original IAC-18 cultivar for control. From 1990 onwards seven trials were carried out under irrigation and normal rainfall conditions in several locations of the various regions where wheat is cultivated in São Paulo State. Under drought conditions the following trials were carried out: Maracai (1990 and 1991, Zone A); Candido Mota (1990, Zone A); Capão Bonito (1992, Zone B). The following trials were carried out under spray irrigation: Mococa (1991, Zone H); Tatuí (1992, Zone D) and Campinas (1992, Zone H).

A randomized complete block design with three replications was used in all the experiments. Each trial was made up of thirty plots with six three-meter rows spaced at 0.20 m. A lateral spacing of 0.60 m was used between plots. Eighty viable seeds were sown per meter of row, equivalent to 1440 seeds in a harvest area of 3.6 m² of each plot.

The following data were collected:

- **plant height:** measured in the field, at the beginning of maturity, as the distance from the soil level to the tip of the ear, excluding the awn.
- **time from emergence to maturity:** days from emergence of the seedling to maturity and classified as

early, from 100-120 days; medium, from 121 to 130 days and late, more than 130 days.

- **stem and leaf rust:** assessment of these diseases, caused respectively by *Puccinia graminis f. sp. tritici* and *P. recondita*, was made by general observation and observation of the stem and upper leaves of each plant at the beginning of maturity under natural conditions for infection. The modified Cobb scale to assess the resistance in the International Spring Wheat Nursery (Schramm *et al.*, 1974) was used. The scale goes from 0 to 99% of infected leaf area, complemented with the reaction types: S = susceptible (large uredosore coalescent, without either necrosis or chlorosis); MS = moderately susceptible (moderate uredosore); M = intermediate (several types of reaction); MR = moderately resistant (small uredosore) and R = resistant (very small uredosore, surrounded by necrotic areas).

- **leaf spot:** this disease, mainly caused by *Helminthosporium sativum*, was assessed at the adult plant stage under natural conditions using a scale of 0 to 99% of infected area where zero is considered immune, 1 to 5% resistant, 6 to 25% moderately resistant; 26 to 50% susceptible and 51 to 99%, highly susceptible (Mehta, 1978).

- **mildew:** assessment for mildew caused by *Erysiphe graminis f. sp. tritici* was carried out in every plot at the adult plant stage, under natural conditions, using a scale of 0 to 99% of infected area where 0 is considered immune, 1 to 5%, resistant, 6 to 25%, moderately resistant, 26 to 50%, susceptible and 51 to 99%, highly susceptible (Mehta, 1978).

- **lodging:** the percentage of lodged plants in each plot was assessed visually near maturity on a scale that varied from 0 (no lodging) to 100% (all plants lodged).

- **grain yield:** weight in grams of the total grain yield of each plot, transformed to kg/ha.

- **ear length:** the average length in centimeters of ten ears randomly chosen in each plot, excluding the awns.

- **spikelets:** the average number of spikelets from ten randomly chosen ears in each plot.

- **grains per ear:** average number of grains counted on ten randomly chosen ears in each plot.

- **grains per spikelets:** this was calculated by dividing the total number of grains from ten randomly chosen ears by the total number of spikelets in each plot.

- **weight of 100 grains:** the weight in grams of 100 grains randomly taken from the total yield of each plot.

The last five characteristics were only assessed in the trial at Tatuí in 1992.

Statistical analyses

Grain yield and plant height data were analyzed first, separately for the irrigated and without irrigation experiments. Once the homogeneity of the variances between the two conditions had been observed, the uniform planning of the seven advanced trials allowed a balanced joint analysis for the two conditions. The analysis of variance was used to test by the F test the presence of the main effects of genotypes, irrigation and experiments and the interaction between genotype x irrigation. Significant genotype effects were observed and each selected line was compared with the IAC-18 control cultivar using the Dunnet test.

The length of ear, number of spikelets per ear, number of grains per ear, number of grains per spikelet and weight of 100 grain data, scored in the 1992 Tatuí experiment, were analyzed according to the planned randomized complete model. The means of the selected progenies were compared with those of the control (IAC-18) using the Dunnet test.

RESULTS

Sensitivity determination for the choice of gamma ray dosage

Table I shows the results of relative seedling height at 20 days after planting (control height was taken as 100.0).

As expected for the M_1 plants in which the physiological effects of the gamma rays were observed, there was a decrease in plant height as the irradiation

Table I - Seedling height¹ in the M_1 generation of the IAC-18 wheat cultivar obtained from the irradiation of the seeds with gamma rays.

Dose (krad)	Height
0	100.0
10	91.2
15	92.0
20	89.2
25	78.0
30	70.5
35	63.0
40	45.5
45	23.3
50	37.8

¹ Mean height compared with the control, IAC-18, taken as 100.0, 20 days after planting.

doses increased. In the light of these results it was decided to use a 40 krad dose which produced a decrease of about 50% in the seedling height in the M₁ generation.

Selecting progenies of IAC-18

Progeny selection, according to the previously described objectives and criteria, started in the M₂ generation. In 1986, 488 plants were selected, and their offspring observed in the field in 1988, when 40 of them were included in the preliminary yield trials carried out in 1990. The nine lines that went to the advanced yield trials came from this preliminary test.

Joint analysis of grain yield and plant height and results of average lodging from the advanced yield trials

To simplify the presentation of the results in face of the large volume of collected data, only the results of the joint analysis were presented. Table II shows the joint analysis results of the seven advanced trials under normal rainfall and irrigation conditions.

The variability coefficients were acceptable. In all cases the F value for trials was significant, indicating the presence of significant year and location effects. For grain yield, the F value indicated significant differences, and lines 8 and 9 had lower grain yield than the original cultivar. The genotypes behaved similarly under irrigation or drought conditions, as indicated by the nonsignificant interaction (genotype x irrigation) F value. However, as expected, a significant F value for irrigation effects was observed. Similarly there was no interaction between plant height and irrigation, but significant main irrigation effects were observed. In spite of the occurrence of shorter plant height for all progenies, significant reduction in height was only observed in progenies 13, 14, 15 and 16. All the lines had lower lodging scores than the control IAC-18.

Other agronomic characteristics of the assessed genotypes in the advanced yield trials

Table III shows, according to the scale previously described, the minimum and maximum scores of leaf spot, mildew, leaf and stem rust, observed in the trials where these diseases occurred.

According to the criteria adopted, all the genotypes were susceptible to leaf spot, because there was an infected area of between 26 and 50% in at least one trial.

Table II - Yield means¹, plant height and lodging of the nine progenies selected from the irradiated seeds treatment compared with the original IAC-18 cultivar².

Genotypes	Yield (kg/ha)	Height (cm)	Lodging (%)
IAC-18	2.484a	86a	42
L-8	1.828b	82a	22
L-9	2.044b	83a	14
L-10	2.274a	80a	14
L-11	2.142a	83a	32
L-12	2.050a	83a	8
L-13	2.300a	78b	14
L-14	2.258a	75b	20
L-15	2.325a	76b	14
L-16	2.291a	78b	18
F (genotypes)	3.17**	4.51**	
F (genot. x irrig.)	1.14 ns	0.91 ns	
F (irrigation)	747.67**	279.00**	
F (experiments)	20.10**	23.32**	
CV%	13.29	5.83	

¹ Mean of seven experiments carried out in various years and locations under irrigation and without irrigation.

² Progeny means were compared with those of the IAC-18 cultivar using the Dunnet test. Progeny means followed by the same letters as IAC-18, in the analyses where the F values were significant, were not significantly different from the control.

Table III - Minimum and maximum disease incidence in the selected progenies and the original IAC-18 cultivar, assessed in the seven trials.

Genotypes	Leaf spot	Mildew	Rust	
			Leaf	Stem
IAC-18	10-40	0-40	tS-30S	0-20S
L-8	10-40	30-40	0	0-10S
L-9	20-40	0-30	0-5S	5S-20S
L-10	20-40	5-40	0-10S	5S-10S
L-11	10-40	40-50	0-10S	0-10S
L-12	10-40	40-80	0-10S	0
L-13	10-50	20-40	0-10S	0
L-14	10-40	5-40	5S-10S	0-5S
L-15	20-50	20-50	0-10S	0
L-16	10-40	10-40	0-ts	0-5S

Progeny 12 was considered highly susceptible to mildew, because an incidence of over 50% in at least one location was observed. The other progenies were considered susceptible, because of an incidence of between 26% and 50% in at least one location.

All progenies were considered resistant to leaf rust at the adult plant stage. Progeny 8 was immune and the other had maximum reaction, varying between tS and 10S. Under the same conditions, the original IAC-18

cultivar used as a control was susceptible with a maximum reaction of 30S.

Progenies 12, 13 and 15 were immune to the stem rust causing agent and progenies 14 and 16 were resistant (maximum degree of infection of 5S) when compared with the control cultivar IAC-18 (20S) under the same conditions.

Table IV shows the yield component results, observed in the 1992 Tatuí trial under irrigation. For all the assessed characteristics there were progenies with significant alterations compared with the control. In the case of ear length, progenies 8, 9 and 11 had higher, progenies 13 and 15 had lower and the others had similar means to the control. Five of the progenies had lower means for number of spikelets per ear than the control and the others had similar values. For the other assessed characteristics, the means were either statistically similar or higher than the original cultivar.

Table V shows the mean length of the roots of the selected lines and of IAC-18, measured after 72 h of growth in the treatment solutions containing four aluminum concentrations. According to the criteria adopted for this type of assessment (Camargo and Oliveira, 1981), progeny 12 did not show root growth at 3 mg/l and was considered susceptible to aluminum toxicity. The other genotypes were considered tolerant with root growth in 6 mg/l Al³⁺. Progenies 8, 9, 10, 11 and 15 were considered very tolerant because they showed root growth after 48 h in solutions with 10 mg/l Al³⁺.

The life cycles of the different genotypes were not presented, because although there was a variation in earliness (100 days) to moderately earliness (110 days), according to the experiment location and year, no significant differences were found among the selected progenies and IAC-18 cultivar.

DISCUSSION

Height and M₁ generation plant survival are some of the most widely used criteria for selecting the dose for mutation induction in cereals (Gaul, 1977). The first parameter is commonly used to select the dosage due to the high correlation between height and survival. The criterion adopted varies from author to author, but in general gamma ray doses that cause 30 to 50% reduction in seedling height are recommended (Konzak and Mikaelson, 1977). In the present study, the dosage

Table IV - Mean¹ data for ear length, number of spikelets per ear, number of seeds per ear, number of seeds per spikelet and weight of 100 seeds of the nine progenies selected from the irradiated seeds and IAC-18, assessed in the 1992 Tatuí experiment.

Genotypes	Ear length (cm)	Spikelets per ear (number)	Seeds per ear (number)	Seeds per spikelet (number)	Weight of 100 grains (g)
IAC-18	7.74a	19.1a	25.2a	1.31a	4.47a
L-8	8.80b	19.7a	35.2b	1.77b	5.06b
L-9	9.11b	20.6a	37.4b	1.81b	4.91b
L-10	8.50a	20.0a	37.4b	1.85b	4.93b
L-11	9.05b	19.5a	34.5a	1.77b	5.06b
L-12	8.49a	16.5b	26.7a	1.61a	5.14b
L-13	6.25b	16.9b	29.3a	1.73a	4.35a
L-14	6.42b	16.6b	31.1a	1.88b	4.42a
L-15	6.40b	16.6b	27.8a	1.66a	4.36a
L-16	6.30b	16.3b	27.4a	1.66a	4.39a
F (genotypes)	40.96**	19.5**	3.5**	3.3**	17.0**
CV%	4.98	3.8	12.7	9.0	3.0

¹ The progeny means were compared with those of the IAC-18 cultivar by the Dunnet test. Progeny means followed by the same letter as IAC-18, in the analyses where the F values were significant, were not significantly different from the control.

which caused about 50% reduction in plant height was used. The results obtained with the selection of several interesting mutants confirmed that the dosage was correct.

There are various methods for selecting populations derived from mutagenic treatments for later mutant selection (Ukai and Yamashita, 1974). One of the methods recommends picking one seed from each M₁ plant to make the M₂ generation. For this method to be efficient, however, it is necessary to treat a large

Table V - Mean root length of the selected lines and the control IAC-18 measured after 72 h of growth in complete nutritive solution, which was followed by 48 h of growth in aluminum solution.

Genotypes	Aluminum concentration (mg/l)			
	0	3	6	10
	mm			
IAC-18	57.1	21.6	7.4	0.0
L-8	53.6	18.7	14.4	2.5
L-9	48.5	26.1	7.4	5.7
L-10	54.7	21.6	10.9	0.8
L-11	53.6	26.4	8.3	1.8
L-12	78.9	0.0	0.0	0.0
L-13	27.4	6.3	2.8	0.0
L-14	35.4	5.2	3.1	0.0
L-15	49.2	6.8	3.2	0.5
L-16	33.0	6.8	1.3	0.0

number of seeds. In the present work 160,000 seeds were irradiated, allowing this method to be used. To avoid the work of individually harvesting one seed from each M_1 plant, a sample corresponding to double the number of treated seeds was taken for the constitution of the M_2 generation, where selection should start. As seen in the results, this method allowed selection of the desired mutants.

The data obtained for plant height in the seven advanced trials (Table II) indicate that in four progenies mutants with lower plant height were obtained, confirming the results from previous selections. These progenies also showed reduction in lodging percentage, thus indicating the benefits of the reduced height. As has been proved in several mutants obtained from wheat (Scarascia Mugnozza, 1965) among those with resistance to lodging, 50% show a reduction in plant height. This indicates that the lower height is an important factor, but not the only one responsible for reduction of lodging, and that, in certain cases, structural aspects of the stalk should be analyzed.

Four of the shorter progenies did not show a significant reduction in grain yield (Table II) and had the same tolerance to Al^{3+} as the original IAC-18 cultivar (Table IV). Progenies 8 and 9, in spite of being more tolerant to Al^{3+} toxicity than IAC-18 cultivar (Table IV), had significant grain yield reduction and progeny 12 maintained the yield level of the original cultivar but became susceptible to Al^{3+} .

There were significant alterations in various progenies in the five yield components assessed in the 1992 Tatuí experiment. For ear length and number of spikelets per ear, significantly lower progeny means were observed relative to the control, while for the other traits the alterations resulted in greater or similar means. In spite of these results and of the fact that these data are based on only one experiment, it is clear that great care must be taken in assessing mutants because mutant selection for one trait may alter others.

As for disease resistance, which was the other objective in this work, all lines were more resistant to the causal agent of leaf rust and eight had a better resistance reaction to stem rust, when compared with the control. Similar to the height reduction example, there were progenies with greater resistance, with the basic characteristics unaffected. This was the case of progeny 10, which is resistant to leaf rust and retained the same yield, plant height and aluminum tolerance of IAC-18. But other mutants were altered drastically, such as mutant 8, which in spite of being immune to rust, had a significantly lower yield. In cereals there is a classic example of a mutation due to a recessive allele, which resulted in immunity to all the mildew races but was

found to be associated with other mutant genes conditioning reduced yield and that made it difficult to use in genetic breeding programs (Konzak, 1984).

Among the resistant mutants, four stood out (progenies 13, 14, 15 and 16) because they united resistance to leaf and stalk rust and reduced plant height, without significant alteration in yield and aluminum tolerance.

These results in which some mutants are less and others more altered in relation to the original cultivar can be expected in mutation induction. Selection can be controlled but not the induction. The use of large populations is, therefore, justified when using this technique to increase genetic variability in plant breeding. As observed by Donini *et al.* (1984), at the beginning of mutation induction studies, the first populations used were relatively small. Presently, the use of large population allows the selection of various mutants with the same phenotype. However, such mutants differ genetically, allowing the choice of the best from an agronomic point of view, as in this study. Thus, the use of large populations is indispensable for mutation induction, to modify a "defect" in a given cultivar without altering its other basic characteristics.

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RESUMO

Objetivou-se a redução na altura da planta e resistência às doenças através da indução de mutação por raios-gama no cultivar de trigo (*Triticum aestivum* L.) IAC-18. Sementes foram irradiadas com 40 krad e a partir da geração M_2 iniciou-se a seleção. Nove linhagens selecionadas foram comparadas com o cultivar original IAC-18 durante três anos, em sete ensaios avançados realizados em vários locais, em condição de irrigação e sequeiro. Quatro das linhagens apresentaram significativa redução na altura e menor porcentagem de acamamento, mantendo inalteradas a produção de grão e a tolerância ao alumínio. Todas as linhagens selecionadas foram mais resistentes ao agente causal da ferrugem da folha e três apresentaram imunidade à ferrugem do colmo. Dentre as linhagens resistentes, algumas não mostraram alterações na produção de grãos e tolerância ao alumínio, mantendo assim as características do cultivar IAC-18, demonstrando-se a utilidade do método para a correção de defeitos em cultivares que já apresentam uma série de boas características agrônômicas.

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