Radiosensitivity of California Mariout barley seeds and determination of the optimal doses for mutation breeding

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ABSTRACT

The locally adapted barley variety California Mariout (C.M) was used to investigate the effect of different gamma ray doses (0, 50, 100, 150, 200, 250, 300, 350 and 400 Gy) from a colbat-60 source on emergence and seedling height and to determine the optimal mutagenic doses required for mutation breeding for improving this barley variety. Prior to irradiation, dry C.M. seeds were equilibrated to a 13% moisture content for higher mutagenic efficiency. The irradiation with gamma ray produced a significant effect on emergence and seedling height compared to the control (0 Gy). At the lower doses up to 200 Gy, emergence % was slightly but not significantly increased. At the higher doses 250, 300, 350 and 400 Gy. emergence % was significantly delayed and reduced scoring 68.9%, 70%, 34.4% and 25.5%, respectively, the control being 90%. The lower doses 50 Gy and 100 Gy produced a radiostimulant effect on seedling height and gave a significant increase in the length of the first leaf of approximately 10.4%. The doses 150 Gy and 200 Gy slightly reduced the seedling height and the high doses 250-400 Gy reduced the seedling height significantly. The correlation coefficient for seedling height was very high (–0.906) indicating an inverse relationship with dose with a regression coefficient of -0.0181. Using the regression equation, the GR10 and GR15 were extrapolated to be 182 Gy and 218 Gy, respectively, and were selected as the optimal mutagenic doses for use in mutation breeding programmes for improving C.M.

INTRODUCTION

Sparsely ionizing radiation has been known for its mutagenic effect on plant species at the turn of the century and has been utilized for the purpose of crop improvement (5, 6, 7, 8). Studies on factors that modify the radiosensitivity of many crop species are well established (2, 3, 12). The most important factors to dormant seed irradiation are oxygen and water content. Their enhancement of gamma rays induced damage can be minimized for higher mutagenic efficiency by adjusting the seed moisture content to 12-14% (9). Mutagens, whether physical (radiations) or chemical, produce three kinds of effects that have significance in plant breeding and genetics. These are physiological damage which is restricted to the first generation (M1) and point (gene) mutations and chromosome mutations which could be passed on to the next generations.

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The extent of the three effects depends largely on dose. Lethality is the main limiting factor for increasing the dose since it increases with increasing dose. Thus, mutagen doses that give low physiological injury but high genetic effects (mutations) are desirable.

There is a correlation in cereals between seedling height after irradiation in the M1 generation and survival on the one hand and M1 mutation frequencies on the other (9). Thereafter, a quantitative determination of the physiological effects in the M1 generation has been a routine procedure in mutation breeding programmes. Such M1 injuries include reduced seedling root and shoot height, delayed and reduced germination and emergence and reduced survival and sterility (number of spikes/ plant, number of seeds/spike and number of fruits and/or seeds/plant). This paper studies the effect of different gamma ray doses from a colbat-60 source on emergence and seedling height of the locally adapted barley variety California Mariout (C. M) and uses the results thus obtained to determine the optimal doses to be used for mutation breeding for improving yield, resistance to drought and resistance to salinity.

MATERIAL AND METHODS

Before irradiation, 250 (gms) of the locally adapted barley variety C.M were placed in a 25cm-dessicator containing a 60% glycerol solution to equilibrate the seeds to a moisture content of 13%. The initial moisture content was measured to be 10.6% using a 3 gm sample that was ground and analysed for water content by an infrared moisture analyser. After 5 days of equilibration, the moisture content of the seeds increased to 13% and the equilibrated seeds were irradiated with 9 different gamma ray doses from a cobalt-60 source (0, 50, 100, 150, 200, 250, 300, 350 and 400 Gy). The dose rate of the cobalt-60 source at the time of irradiation was 10.7 Gy/min. Right after irradiation, seeds were taken to a uniform section of the greenhouse and planted in two experiments to study the effect of gamma rays on emergence and seedling height using the length of the first leaf as a criterion.

Regarding emergence, a CRD design was layed out with 3 replicates for each of the 9 doses. Pots were filled with equal amounts of sandy soil collected from a homogenous site of the experimental field at T.N.R.C. and passed through a 2mm sieve. The irradiated seeds were placed on the surface and covered with a 4cm-layer of sand. Distilled water was used to water the pots to field capacity. Six days after planting, the seeds started to emerge and emergence % was recorded for 6 consecutive days- up to day 11 when readings were fixed.

To study the effect of gamma rays on seedling height the irradiated seeds were sown in rows in boxes filled with sandy soil collected from a homogenous site of the experimental field and passed through a 2mm-sieve. 3 boxes were used, each representing a replicate. Row-row distance was 3 cm and the seeds were sown 1cm deep. The mutagen induced shortening of the germinated plants as measured on the first leaf. Measurements were taken when the first leaf stopped its growth, 19 days after planting, to clear any confusion as to whether the shortening was due to delayed germination or to a real radiation effect. Samples of individual leaves were measured daily to determine wheather the first leaf was still growing.

RESULTS AND DISCUSSION

Table [1] shows the height of the first leaf of C.M seedlings as affected by the different gamma ray doses applied. A significant increase in length of about 10.4% was observed at the lower doses 50 Gy and 100 Gy. This so called radiostimulation effect has been studied by many researchers (10, 14, 15) and the doses producing the highest levels of stimulation have been successfully used by many breeders worldwide to increase productivity, resistance to many diseases, protein content and to improve the quality of a lot of crops such as potatoe, tomatoe, barley, wheat, rice, peas, beans,...etc. This radiostimulation effect is due to radiation-induced increase in DNA and RNA content of meristems. It may also be produced by radiation-stimulated physiological and biochemical cell processes manifested in increased permeability of cell membranes, increased mobility of food reserves to growing regions and accelerated germination and growth Radiostimulation also enhances the process of photosynthesis and the accumulation of its products as well as increases growth and development of plants resulting in an early crop (1, 13).

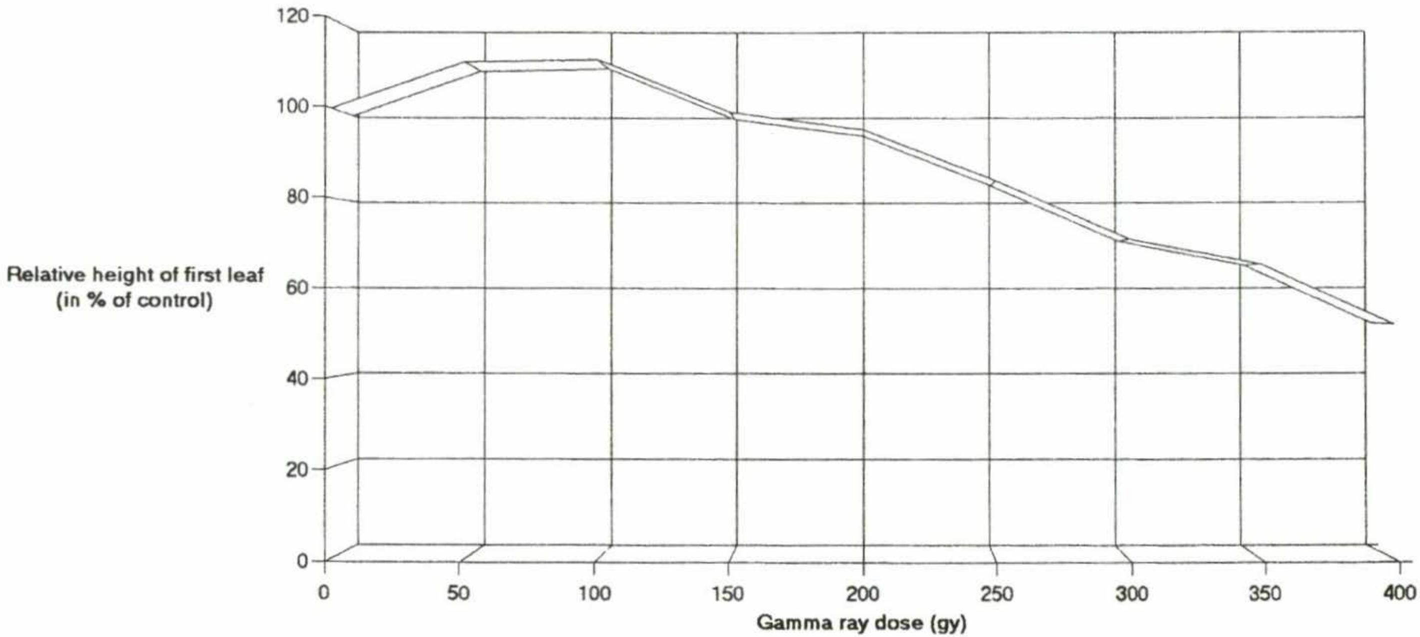
Table 1 – The effect of different gamma ray doses on the first length of C.M seedlings. The coefficient of correlation, the coefficient of regression and the intercept for the equation of regression are shown.

gamma ray doses (Gy)	Length of first leaf (cm)	Coefficient of correlation	Coefficient of regression (b)	Intercept (a)
0	12.87 b			
50	14.20 a			
100	14.27 a			
150	12.77 b			
200	12.27 b	-0.906	-0.0181	14.892
250	10.80 c			
300	9.13 d			
350	8.40 d			
400	6.67 e			

Means followed by the same letter are not significantly different at alpha = 0.05 according to Duncan's multiple range test.

The doses 150 Gy and 200 Gy caused a decrease in seedling height, though not significantly. At doses of 250 Gy and higher, the physiological damage caused a highly significant decrease in seedling height, the damage being more pronounced as the gamma ray dose increased. This trend is represented graphically in graph[1] and is typical of a lot of crops studied (3, 9, 12). This highly significant negative correlation observed had a coefficient of -0.906. The regression of seedling height on gamma rays

Graph 1- effect of different gamma ray doses on seedling height of C.M barley seeds.



was highly significant and scored a coefficient of -0.0181 and an intercept of 14.892, making a linear regression equation of y = 14.892–0.0181*x. This equation was used to extrapolate the GR50, the dose producing 50% reduction in seedling height, and was found to be 469 Gy, a finding consistent with previous findings by other investigators. Makaelsen and Brunner, using gamma rays, found the GR50's of 7 different barley varieties to range from 330-450 Gy (11). Gopal-Ayengar, et al. found 500 Gy as the GR50 of the barley variety Himalaya 620 (8). For quantitatively inherited traits such as yield and physiological traits like resistance to drought and salinity, the dose range GR10-GR20 is recommended to be the optimal one for crop improvement (9). The GR10 and GR15 were extrapolated to be 182 Gy and 218 Gy, respectively, and will be the doses used for C.M improvement through mutation breeding. The GR20 dose was 254 GY and will not be used for mutation breeding due to its significant effect in reducing emergence % as will be seen next.

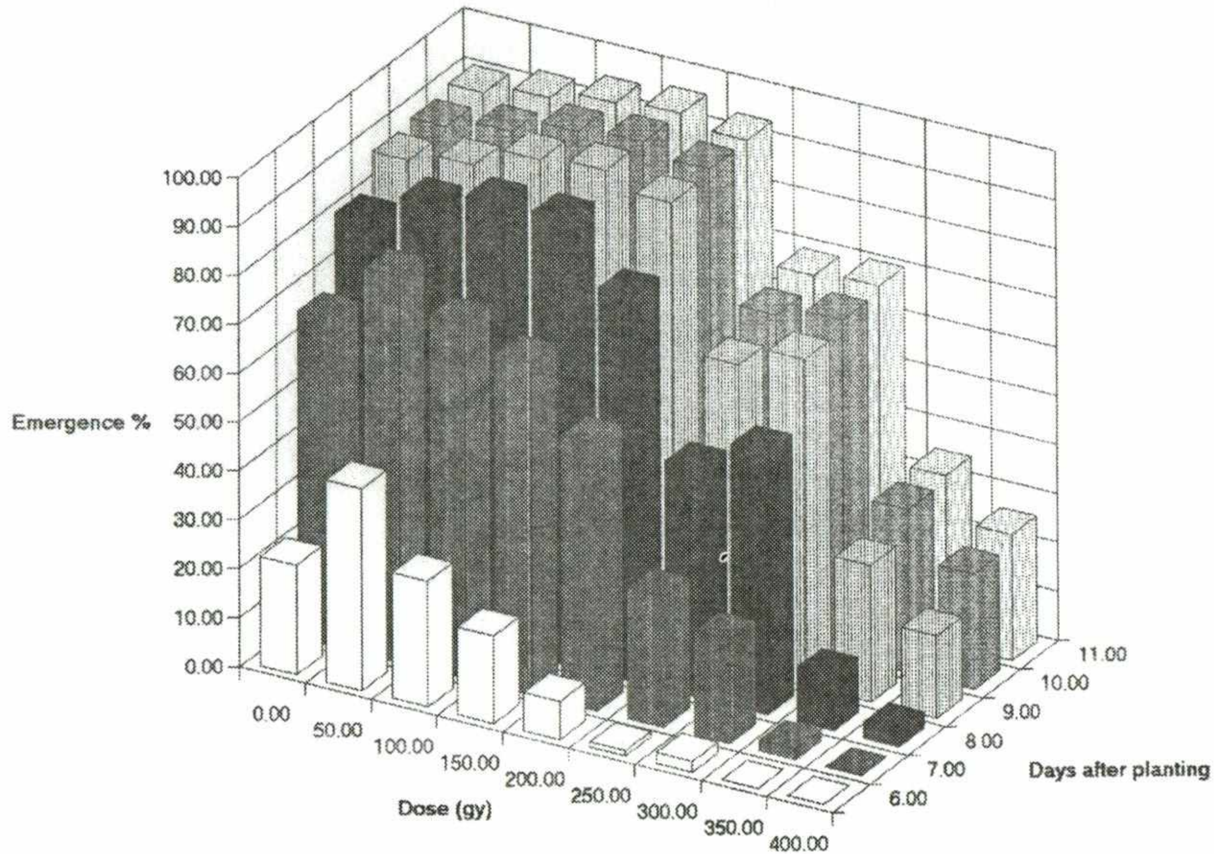
Table [2] shows the effect of gamma ray doses on the emergence % of C.M. It can be seen that as the irradiation dose was increased emergence % was significantly delayed and reduced at the high doses. The control material (0 Gy) recorded 81% emergence on the eighth day after planting whereas the high doses 250, 300, 350 and 400 Gy recorded just 46.6%, 53.7%, 11% and 3.3%, respectively. Upon completion of emergence on day 11, when emergence was fixed for all doses, the control was 90% emerged whereas the high doses were 68.9%, 70%, 34.4% and 25.6% emerged, respectively. Graph [2] plots these data of table [2] for graphical representation. The lower doses 50, 100, 150 and 200 Gy resulted in a slight increase in emergence over the control, but not significantly.

Table 2 – The effect of different gamma ray doses on the emergence % of C.M barley seeds.

gamma ray dose (Gy)	Days after planting 6	7	8	9	10	11
0	22.23	66.67	81.10	87.77	88.87	90.00 a
50	41.13	78.87	87.80	89.00	91.13	92.23 a
100	25.53	72.23	91.10	94.43	94.43	94.43 a
150	17.80	67.77	91.13	95.57	95.57	95.57 a
200	7.77	54.47	78.90	92.20	93.33	93.33 ab
250	1.10	26.67	46.67	62.23	66.67	68.90 bc
300	2.20	21.10	53.33	66.67	68.90	70.00 c
350	0.00	2.23	11.10	27.77	33.33	34.43 d
400	0.00	0.00	3.33	16.67	23.37	25.57 d

Means followed by the same letter are not significantly different at alpha = 0.05 according to Duncan's multiple range test.

Graph 2-effect of different gamma ray doses on emergence % of C.M barley seeds.



LITERATURE CITED

1. Al-oudat, M. 1987. The influence of preirradiation of seeds on growth development and yield of barley and wheat plants grown in Syrian Arab Republic. Radiobiology 27:181-185.
2. Conger, B. V., M. J. Constantin, J. V. Carabia. 1972. Seed radiosensitivity: wide range in oxygen-enhancement ratio after gamma irradiation of eight species. Int. J. Radiat. Biol. 2:225-235.
3. Donaldson, E., R. A. Nilan, C. F. Konzak. 1979. The interaction of 02, radiation exposure and seed water content on gamma irradiated barley seeds. Environ. Exp. Bot. 79:153-164.
4. Gopal-Ayengar, A. R., N. S. Rao, B. Y. Bhatt, K. B. Mistry, D. C. Joshua, R. G. Thakare, 1972. Neutron irradiation of seeds III. IAEA, Vienna., 1972. p: 1-12.
5. IAEA, Vienna. 1981. Induced mutations-A tool in plant research. Proceedings of a symposium, Vienna, 9-13 March 1981.
6. IAEA, Vienna. 1972. Induced mutations and plant improvement. Proceedings of a study group meeting, Buens Aires, 16-20 Nov. 1970.
7. IAEA, Vienna. 1978. Seed protein improvement by nuclear techniques. Proceedings of two research co-ordination meetings, Baden, 28 Mar-1 Apr, 1977 and Vienna, 26-30 Sept, 1977.
8. IAEA, Vienna. 1983. Induced mutations for disease resistance in crop plants II. Proceedings of a research co-ordination meetings RIS, 15-19 June 1981.
9. IAEA, Vienna. 1977 Manual on mutation breeding-Second edition.
10. Kuzin, A. M., M. E. Vagabova, M. M. Vilenchik and V. G. Gogavadze. 1986. Stimulation of plant growth by exposure to low level gamma radiation and magnetic field, and their possible mechanism of action. Envir. Exp. Bot. 26:163-167.
11. Mikaelsen, K., H. Brunner. 1968. Effect of fast neutrons and gamma radiation on seedling shoot and root height of barley varieties. Neutron Irradiation of seeds II, IAEA, Vienna, 1966. Technical reports series No. 92:79-82.
12. Mikaelsen, K., J. Kramer. 1972. Effects of water content, oxygen and metabolic state on genetic effects of fast neutrons and gamma radiation of barley seeds. Neutron irradiation of seeds III. Technical reports series No. 141:59-61.
13. Saleh, S., and K. El-shoney. 1974. Effect of grain irradiation with gamma rays on growth, nitrogen content and yield of wheat plants. Egypt. J. Bot. 17. No. 1:27-33.
14. Sax, K. 1963. The stimulation of plant growth by ionizing radiation, Rad. Botany 3:179-186.
15. Shull, C. A. and J. W. Mitchell. 1933, Stimulative effects of x-rays on plant growth. Plant. Physiol. 8:287-296.

الحساسية الإشعاعية لحبوب الشعير «كاليفورنيا مريوط» وتحديد الجرعات المناسبة لتربية النبات بالتطفير

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المستخلص

تم استخدام صنف الشعير كاليفورنيا مريوط (C.M)، المتأقلم محلياً، لدراسة تأثير جرعات مختلفة (0، 50، 100، 150، 200، 250، 300، 350، 400 Gy) من أشعة جاما من مصدر الكوبلت 60 على الإنبات وطول البادرة، وبالتالي تحديد الجرعات المطفرة المناسبة لتحسين هذا الصنف. قبل عملية التشعيع تم ضبط رطوبة الحبوب إلى 13٪ من أجل الحصول على فعالية أعلى للتشعيع. وقد أثر التشعيع بأشعة جاما معنوياً على الإنبات وطول البادرة الأولى، مقارنةً بالشاهد (0 Gy). عند الجرعات المنخفضة (50--200 Gy) كان هناك ارتفاع طفيف في الإنبات، ولكن عند الجرعات المرتفعة 250، 300، 350، و400 Gy كان الإنبات متأخراً ونسبته أقل معنوياً حيث سجل 68.9٪، 70٪، 34.4٪ و25.5٪، على التوالي، بينما كانت نسبة إنبات الشاهد 90٪. الجرعتان المنخفضتان 50 و100 Gy أعطتا تأثيراً تحريضياً على طول البادرة الأولى، حيث زادت معنوياً بمقدار 10.4٪. والجرعتان 150 و200 Gy سببتا انخفاضاً بسيطاً في طول البادرة، بينما سببت الجرعات العالية 250–400 Gy نقصاً معنوياً في طول البادرة. وقد كان معامل الارتباط لطول البادرة مرتفعاً (‎-0.906) مبيناً علاقة عكسية بالجرعة بمعامل انحدار ‎-0.0181. من خلال معادلة الانحدار تم استنتاج الـ GR10 والـ GR15 وكانتا 182 و218 Gy على التوالي، وقد تم اختيارهما على أنهما الجرعتان المناسبتان لعملية تربية صنف الشعير .C.M بالتطفير لغرض تحسين خصائصه.

(1) مركز البحوث النووية – تاجوراء. إدارة تطبيقات النظائر المشعة. ص.ب.: 30878.