MOLECULAR BIOLOGICAL STUDIES ON THE EFFECT OF GAMMA RAYS AND MUTAGENIC CHEMICALS ON DROUGHT RESISTANCE IN PLANTS

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ABSTRACT

Nahla Hamid Eldin Mohamed Elsaid. [Molecular Biological Studies On The Effect Of Gamma Rays And Mutagenic Chemicals On Drought Resistance In Plants] Doctor Philosophy Degree in Science, Botany Department, Faculty of Science, Ain Shams University (2004).

Different doses of gamma rays and chemical mutagen have been applied as seed treatment of four plant strains. Seed treatment with 60 Gy gamma irradiation and 0.001 M Na-azide were selected to be used with the two maize strains G4 and Rg11 for further experimentation. Growth parameters and endogenous hormone levels indicated that strain Rg11 is more suitable than G4 under normal irrigation, whereby G4 did better performance under drought stress. The changes at the molecular levels showed variations in the protein banding patterns and the nucleic acid DNA, in response to the applied mutagenic treatments. It has been recommended to cultivate the maize strain G4, in consequence to seed irradiation with 60 Gy gamma rays, for drought resistance.

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PREFACE

Crop yields increased dramatically in the 20th century as recorded in the world averages. In the next century, there is a number of challenges to be faced in order to maintain the necessary food production. These include an estimated increase in the world's population to around 8 billions by 2020 and global warming causing more frequent and severe fluctuations in climate, thus increasing the chance of crop failures. Strictly limited availability of land, and shortage of the water necessary to support crop growth with irrigation are also predicted (FAO / IAEA Division, 1999).

Crop plants are the direct or indirect source of virtually all our food. Crop plants, unlike animals, stay in one place and are therefore, controlled by the environment in which they find themselves. As a consequence, they have evolved a complex genetic system, which enables them to adapt the change in environment in order to complete their life cycle. The productivity of plants is greatly affected by environmental stresses such as drought, salinity, low and high temperature (Miflin, 2000). The genetic improvement of stress tolerance is an urgent need for the future of agriculture.

Maize (*Zea mays* L **.**) has long been utilized as an important human and animal food source. In Egypt, there is a wide gap between cereal crop production and consumption. To decrease this gap, we must increase the land cultivated with cereal crops, but this new land has many conditions of environmental stresses. Rapid methods are needed to identify germplasm potential for yield under different environmental stresses and soil conditions.

Mutations are the source of all hereditary variation and provide the basis of evolution. Physical and chemical mutants were used in several crop species to induce variation in quantitative and qualitative traits. Ionizing radiation induces various alterations to morphological and physiological processes in plants (Casarett, 1968) and can produce modifications on the DNA bases as those induced by Fe^{3+} and H_2O_2 *in vitro* (Aruoma *et al.*, 1989 a & b).

The growth habits and physiological properties of plants may differ markedly under different regimes of light, gravity, temperature, humidity and salinity. In this respect, hormones have long been known as important internal mediating signals in plants and the components of the underlying cellular machinery were identified and characterized (Grill and Hummelbach, 1998; Solano and Ecker, 1998; D'Agostino and Kiebar, 1999; Trewavas, 2000). Certain mutations can simultaneously influence the response to more than one hormone or to an altered physical parameter, including drought (Wilson *et al.,* 1999a; Benudoin *et al.,* 2000; Ghassemian *et al.,* 2000).

Molecular markers such as random amplified polymorphic DNAs (RAPDs) (Welsh and McClelland, 1990; Williams *et al.,* 1990) and restriction fragment length polymorphisms (RFLPs) (Grodzicker *et al.,* 1974) have been used in studying phenomena (such as heterosis) and showed an excellent potentiallity to assist selection of quantitative traits (Stuber, 1992), besides their use in the construction of genetic maps in maize (Helentjaris *et al.,* 1986; Hoisington and Coe, 1990).

INTRODUCTION

1. Growth Criteria

1.1. Vegetative characters

1.1.1. Effect of environmental drought stress

Growth criteria are known to be affected by environmental stresses and there are obvious relationships between vegetative characters and the plant yield. Fischer *et al.* (1989) used three levels of soil moisture to detect the most suitable for improvement of the yield of maize under drought. The experiment was carried out under the same conditions for three successive cycles. They studied the traits connected with drought like leaf and stem extention growth, and rate of male and female flowering and yield. They found that there were significant changes in the drought adaptive traits, but there was no significant change in days to-flowering. Grzesiak (1990) also studied the response of different maize genotypes to drought and it's relation with the leaf area, dry matter accumulation and grain yield. The results showed a drop in growth parameters and consequently yield with the reduction of soil water content. Sobrado (1990a) and Zemanek (1992) studied the relation between drought and the leaf area and grain yield in maize and barley. They concluded that in maize, drought caused a decrease of the leaf area, grain size and number. In barley, there was a positive correlation between the area of the three top leaves and both grain number and grain yield under all regimes. Sinclair *et al. (*1990) also found a linear correlation between biomass gain and grain yield of maize under water stress. Sobrado (1990b), Machiaria (1990) and Cramer and Daniel (1991) showed that water stress affected leaf elongation by action on leaf osmotic potential and turgor pressure. Frederick *et al.* (1990) and Sobrado (1990c) studied the action of drought stress in maize on carbon and nitrogen assimilation and their reflection on the total biomass and dry weight of vegetative parts. These parameters were markedly decreased as a result of drought stress. A similar conclusion was also attained by Nesmith (1991) who studied the whole plant responses to drought and the action of water deficits at various growth stages. He revealed that the yield losses are greatest when the water deficit is prevailed during grain filling. Reduction in the extension of internodes, leaves and size of ears, during the deficit period, was primarily attributable to decreased growth rate rather than shortened growth duration. Also, he concluded that above ground biomass accumulation was less under deficit water condition at all growth stages and was largely attributable to reduced production of leaf area or increased loss of green leaf area due to early senescence and leaves rolling. Bolanos and Edmeades (1993) suggested that drought stress coincided with flowering and affected harvest index, yield stability, and grain yield. Calavache *et al.* (1995) identified the specific growth stages of maize crop at which the plant is less sensitive to water stress so that irrigation can be ommitted without a significant decrease in yield, specified flowering and yield formation stages. Abdel-Tawab *et al.* (2002) studied the relationship between morphological performance, yield components, and drought-stress tolerance of eight Egyptian bread wheat. There were significant differences in the area of flag leaf, spike criteria, total biomass and grain yield / plant between the control plants and those under drought. In general, higher values were recorded in the control plants, as compared with those under drought.

1.1.2. Effect of mutagenic agents

Chemical and physical mutagenic agents usually cause changes in the vegetative characters by induction of mutations. In this connection, gamma rays represent one of the important physical mutagenic agents. Jaywardena (1988) used gamma rays for induced mutation to improve the characters and productivity of many plants (rice, maize, bean, cowpea and potato). Balan *et al.* (1989) treated some pollen and grains of maize by gamma rays. The resulted mutant showed changes in height, ear size, number of grain rows and yield. In addition, it was also resistant to cold, drought and lodging. Diaconu (1993) used gamma radiation to induce mutation in the endosperm structure of sugar corn. Gautam (1998) found that 10Krad gamma rays gave the highest mutation frequency in wheat. Four mutants were evaluated in M_3 and M_4 generations for grain yield / plant, plant height, number of tillers / plant, spike length, productivity of spikelets / spike and number of grains / spike, as well as the weight of 1000 grains.

Despite the induction of mutations, many workers studied the effects of radiation on growth criteria and yield of many plants. For example, Narimanove and Korytov (1996) and Narimanove *et al.* (1997) studied the action of gamma rays on the growth of some crop plants (wheat, barley, peas, maize, and *Cucumis melo*). The germination potential, root development and subsequent seedling growth were increased as a result of irradiation of dry seeds by a relatively low dose (10-20 Gy) gamma rays. Rabie *et al.* (1996) also irradiated seeds of faba bean with gamma rays, using 1, 2, 4 and 10 K rad doses, and studied their effects on growth and pod shedding. The results revealed significant increases in germination percentage, seedling length and relative growth rate at low radiation doses (1, 2, 4 Krads). The same treatments also induced high yield and low percentage of pod shedding. The dose 4 Krad was the most effective, whereby higher doses (8 and 10 Krad) led to a significant reduction in the growth characters concomitant with high shedding percentage. Wang *et al.* (1998), subjected the seeds of multiple-row ear of a maize hybrid to gamma ray irradiation. It could be concluded that gamma rays induced not only variation in characters and physiological damage, but also resulted in the production of two maize germplasm materials with good performance such as multiple-row cobs with compact conformation and disease resistance, Abdel-Tawab *et al.* (1998a and 2002) also studied the effect of gamma rays, alone and combined with drought stress, in maize and wheat. They concluded that irradiation positively affected the yield traits, wheareas irradiation combined with drought stress displayed a good performance more than the non-irradiated plants under the same conditions. On the other hand, Abdel-Tawab *et al.* (2001), showed that gamma irradiation and salt stress caused a reduction of some growth traits (plant height, number of leaves, leaf area, stem diameter, fresh and dry weights) and yield of maize. Lal *et al.* (2000a) also reached a similar conclusion in Java citronella (*Cymbopogon winterianus)* and further indicated that a decrease in all plant traits was observed with increase of the radiation dose. On the other side, Chaudhuri (2001) concluded that root and shoot extension of lentil