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Efficiency of energy conversion and growth of gamma irradiated embryos and young seedlings of *Triticum monococcum* L. cultivar Einkorn

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A B S T R A C T

The study was conducted to determine whether the efficiency of energy conversion into growth can be used as an indicator for the determination of the optimal gamma irradiation dosage for mutation breeding. To meet this objective, embryo growth, shoot growth, root growth, mobilization of food reserves, respiration and energy conversion were studied in gamma-irradiated wheat *Triticum monococcum* L. cultivar Einkorn kernels. Kernels were exposed to 50, 150, 250 and 350 Gy and germinated. Kernels were collected 12 h after onset of imbibition and then every 12 h until 168 h. Irradiated seed demonstrated retardation in all parameters, which increased as the gamma irradiation dosage increased. For the most, dosage and time, as well as dosage by time interaction were highly significant. Root growth appeared to be the most sensitive to gamma irradiation, followed by shoot growth, mobilization of food reserves and efficiency of energy conversion. Full recovery of the efficiency of energy conversion took place at 50 Gy, with an increase in inefficiency with an increase in dosage. The point where full recovery of efficiency of energy conversion into growth gives way to incomplete recovery (100 Gy) is in line with the suggested dosages for practical mutation breeding in *Triticum monococcum* L. by the FAO/IAEA and is therefore an ideal indicator for predicting the dosage that will be optimal for plant mutation breeding.

1. Introduction

Triticum monococcum L. is a diploid wheat species that belongs to the *Triticum* polyploid complex. The diploid chromosome number of *T. monococcum* L. is $2n = 2x = 14$ with a genomic constitution of AA (Hilu, 1993). When *T. monococcum* L. kernels germinate, they imbibe water to activate a cascade of metabolic activities to initiate embryo development and growth. These activities include DNA replication, DNA transcription, translation and cell division (Dobrzanska, Tomaszewski, Grzelczak, Rejman, & Buchowicz, 1973; Marcus, Feeley, & Volcani, 1966; Mory, Chen, & Sarid, 1972; Simon & Meany, 1965). During the initial stages of embryo growth, shoots and roots form by means of cell elongation followed by cell division to complete the germination process (Davies & Rees, 1975). When gamma-irradiated kernels germinate, on the other hand, the primary root and first pair of lateral roots are able to emerge without any cell division (Foard & Haber, 1961). However, the emergence of the second pair of lateral roots of gamma-irradiated kernels is dependent on cell division.

Gamma irradiation in particular, is widely used as a modification agent for improving genetic diversity in agriculture for breeding purposes due to its high penetration ability. Gamma irradiation of kernels is regularly performed as a method to induce mutations (Khan, Hassan, Islam, & Biswas, 2013; Majeed & Muhammad, 2010). Its exploitation in agriculture is limited due to uncertainty in the dosage of irradiation which varies for different crops and application (Kurowska, Labocha-Pawloowska, Gnizda, Maluszynski, & Szarejko, 2012; Nepal, Ojha, Sanchez Meador, Gaire, & Shilpakar, 2014). However, mutation induction treatment is convenient, because large quantities of kernels can be irradiated in one session

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and irradiated kernels can easily be stored and shipped (Kodym & Afza, 2003).

It is well established that kernels treated with high dosages of gamma irradiation show retarded development and seedling growth. In the case of moderate stress caused by the irradiation, the adaptability capacity of the plants is preserved and the observed changes are reversible (Stoeva, 2002). However, the precise cause of the retardation is still unknown (Preus & Britt, 2003). Various studies have been undertaken to document the effects of gamma irradiation on the development and growth of seedlings. Different diploid species have demonstrated different sensitivities to gamma irradiation (Wu & Yu, 2001). The effect of gamma irradiation on interphase chromosomes was greatest in *T. monococcum* L., followed by *Aegilops speltoides* Tausch, *A. umbellulata* Zhuk., *A. caudata* L. and *A. comosa* Sm. (Ichikawa, 1969). A positive correlation has also been established between interphase chromosome volumes and the order of sensitivity to gamma irradiation (Donini, Sparrow, Schairer, & Sparrow, 1967). However, a negative correlation was found to exist between DNA content per chromosome and the LD_{50} values for ten species of plants by Baetcke, Sparrow, Nauman, and Schwemmer (1967). In contrast, Degani and Pickholtz (1980) could not find a correlation between nuclear volume and irradiation sensitivity. Furthermore, different wheat cultivars also vary in irradiation sensitivity (Albokari, Alzahrani, & Alsalman, 2012; Borzouei, Kafi, Khazaei, Naseriyan, & Majdabadi, 2010).

A 50% growth reduction of seedling height after acute gamma irradiation of dormant kernels is widely used as a measure of irradiation damage. This reduction in growth is influenced by a series of physiological processes, DNA repair, as well as cell death (FAO & IAEA, 2012). Complete repair of meristematic cells only take place until a certain degree of damage is obtained by the cells. When the damage to the meristematic cells is above a threshold value, incomplete repair takes place. These damaged cells stop dividing and may even be eliminated. Full repair of meristematic cells leads to full recovery and effective energy conversion into growth. However, as the percentage of repaired meristematic cells that participates in the mitotic cell divisions decreases, so does the efficiency of recovery and thus energy conversion into growth decreases. Thus, the aim of this study was to determine whether the efficiency of energy conversion into growth can be used as an indicator for the determination of the optimal dosage for mutation breeding. To test the hypothesis, a number of physiological parameters was measured in developing embryos and young seedlings of *T. monococcum* L. to determine the effect of gamma irradiation. These parameters included growth, respiration and efficiency of energy conversion into growth.

2. Materials and methods

2.1. Kernel preparation for growth experiments

It has been shown that kernel size affects early growth and seedling vigour (Hampton, 1981; Lafond & Baker, 1986). Therefore, the kernels used in this study were selected to fall within 2 mg of the mean weight of 28 mg of a population of kernels of one accession of *Triticum monococcum* ssp. *monococcum* L. cv. Einkorn. To ensure repeatability of the experiments, the moisture content of the kernels was adjusted to 14% prior to irradiation (FAO & IAEA, 2012). The kernels were irradiated with 50, 150, 250 and 350 Gy using a ^{60}Cobalt source at the South African National Blood Services in Roodepoort, Gauteng, South Africa. The irradiated kernels, as well as kernels that were not irradiated, which acted as the control, were placed in the dark between standard germination papers at a constant temperature of 25 °C according to the regulations of ISTA (2015). The kernels were left to germinate and grow for a period of 168 h.

2.2. Embryo/seedling growth, mobilization of food reserves, respiration rates and efficiency of energy conversion into growth

The embryo growth period was regarded as the time period from the initiation of imbibition until 48 h of growth when the primary roots had emerged; at which time germination was considered complete. The seedling growth period, on the other hand, was taken from 48 h until 168 h of growth. Kernels were collected every 12 h, eight kernels at a time, starting at 12 h after the initiation of imbibition. Kernels were then dried for at least 48 h in an oven at 105 °C, after which the dry weights of the embryo, shoot, root and remaining caryopsis, were recorded. The growth rate of an embryo was determined by the rate of increase in dry weight of the embryo, while for a seedling, the growth rate was determined by the increase in shoot and root dry weights. The rate of mobilization of food reserves was calculated as the difference between the initial caryopsis dry weight and the actual dry weight of the caryopsis at a particular point of time. Respiration rate was calculated as weight loss and determined for an embryo as the difference between the initial kernel dry weight and the actual dry weight of the embryo and caryopsis during the period after initiation of imbibition until 48 h of growth. For seedlings, respiration rate was calculated as the difference between the initial kernel dry weight and the actual weights of the shoot, roots and caryopsis during the period from 48 h to 168 h of growth (Bouaziz & Hicks, 1990). The efficiency of energy conversion into growth was calculated as the difference between the combined shoot and root dry weights and the original embryo dry weight. The difference was then divided by the value for mobilization of food reserves.

2.3. Experimental lay-out and statistical analysis

The experimental layout was a randomized complete block design with eight replicates (blocks). A two factor factorial experimental design was followed with six dependent variables with dosage levels of 0, 50, 150, 250 and 350 Gy and time with levels 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156 and 168 h. The effects of the factors dosage and time and the dosage by time interaction were tested using the GLM procedure of SAS software (SAS Institute 2012 North Carolina 27513: SAS Campus Drive, Cary). The least significant means (LSMEANS) of SAS were used to compare the effect of the different dosages on embryo, shoot and root growth, mobilization of food reserves, respiration rates and efficiency of energy conversion into growth at specific p values. To determine which dosages would lead to a 25% and 50% reduction in shoot and root growth, as well as in mobilization of food reserves; a sigmoidal curve was fitted to the data using NLIN Procedure of SAS. The following formula for Sigmoidal curve was used:

$$y = a + \frac{b}{1 + \exp\left(-\frac{x-c}{d}\right)}$$

where y = dependent variable e.g. % root or shoot growth or mobilization of reserve food,

a = intercept
b = maximum % growth
x = dose
c = rate of change (slope)
d = dose at which growth rate reaches its maximum value.

The formula for effective dose to determine 25% and 50% growth

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