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SOME PHYSIOLOGICAL EFFECTS OF NEUTRON-GAMMA RAYS ON CAROB SEED GERMINATION
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ABSTRACT
This paper summarizes a work with neutron-gamma radiation on carob seed germination. Morphological aspects of growth and physiological responses during seedling development have been studied in order to understand the factors influencing plant radiosensitivity.
Dormant seeds have been irradiated with different dose rates - 5,500, 11,000, 22,000 and 88,000 rad - of neutron-gamma radiation. It was observed that seed imbibition was not affected by such radiation. During germination alteration of the normal rate of some vital physiological processes was observed, in spite of the great genetic variability presented by the plant material. Nevertheless the degree of physiological disturbance observed did not enable the further viability of seedlings on the three lower dose rates. It was observed a stimulatory effect on seedling growth, cotyledon area and photosynthesis, under 22,000 rad of dose rate. The seedlings under 88,000 rad, after a previous period of low growth and poor development, have had no further growth.
There are a considerable number of potential applications of ionizing radiation in food preservation. These include, for instance, desinfection of storage plant products such as fruits and seeds. In all cases where food for human consumption is irradiated there is a possibility that adverse changes may occur. On carob dormant seeds it was observed that the extent of such changes depends on dose rate. In some cases the changes were negligible or could be controlled.
The application of this technique to carob storage in a practical and useful fashion seems feasible in the near future.
KEY WORDS: carob, Ceratonia siliqua, neutron-gamma, radiation.

INTRODUCTION

The naturally occurring ionizing radiations (e.g. cosmic rays and those from radioactive elements) have always produced biological effects since time immemorial. The cumulative effects of these radiations might conceivably be of considerable evolutionary significance both for plants and animals. It is obvious that the small increases in background level of radiation due to radioactive fall out, if continued over long periods of time may produce significant biological effects.

Interest in radiobiology resulted from the demonstration that radiation could induce mutations which were important in plant breeding. More recently it was appeared a new field of investigation pertaining to the use of radiation, in food processing and storage.

So, fundamental research in radiobiology is, very important specially because it is not fully understood the mechanisms of radiation damage to plants.

During maturation and storage, carob pods can be attacked by different pests causing damage and decreasing their commercial value (Teio and Almeida, 1980). To prevent this damage it would be interesting to know if we could use radiation before pod storage and seed industrialization. Before this it would be necessary to study the radiosensitivity of carob seeds and to know the extent of uptake in order to evaluate the radiation exposure. In all cases where food for human consumption is irradiated a great care must be taken. It is advisable to study the adverse changes which may occur. The following paper describes briefly the experiments realized in order to study the effect of neutron-gamma radiation on carob seed germination.

MATERIAL AND METHODS

Dormant carob seeds were irradiated in the RPI reactor from LNE GT with 5,500, 11,000, 22,000 and 88,000 rad of neutron-gamma radiation. These dose rates correspond to different times of radiation exposure - 300, 600, 1200 and 4800 s, respectively. In more facility they have received the letters B, C, D and E.

Three months after irradiation carob seeds were treated with concentrated sulphuric acid for 15 min to promote the breaking of dormancy. Following imbibition in tap water during 48 h, selected seeds from all treatments were allowed to germinate in separated plastic pots with wet sterilized expanded clay
at greenhouse conditions with an average temperature of 24°C. One control, A, without irradiation, was allowed to germinate in the same conditions. Germination has been done without any addition of nutrient solution to the seedlings. They were only water pulverized daily.

Once each week during the course of 37 days experiment, a batch of 30 seedlings from each treatment were harvested for growth analyses and photosynthesis determinations.

Cotyledon areas were measured with a LI-3000 Portable Area Meter (LI-COR). Dry weight of plant parts were analysed after overnight drying at 80°C. Total nitrogen content was analyzed by a microkjeldahl procedure. Hypocotyl length values served as an index of growth since root system easily disrupted during harvest. Chlorophyll content of cotyledons was analyzed by Oserol and Titus method (1963). Net photosynthesis was measured on cotyledons placed in a broad-leaf chamber using a portable infrared gas analyser from ADC. Light was supplied from a halogen lamp of 150 W with a photon flux density of 1000 umol. m−2.s−1. Chamber temperature was maintained at 25°C.

The thickness of cotyledons was compared using the specific cotyledon weight, it means the ratio of dry weight and cotyledon area.

RESULTS AND DISCUSSION

The results show that during germination there was an alteration of the normal rate of some vital physiological processes.

After 2 months the growth was completely different in treated seedlings compared to the control. It could be noted that the highest dose rate (88,000 rad) lead to the premature death of the seedlings with the growth allocated only on cotyledons.

Nevertheless germination can occur in all group of seeds (Fig. 1) independently of the dose rate. It is already known that dormant seeds are less radiosensitive than seeds with developing embryos (Casarett, 1968). On contrary, it is generally accepted that the degree of damage is increased as the time is increased between irradiation and planting (Casarett, 1968). It seems that the storage effect of three months did not induced a low seed radiosensitivity. Probably free radicals are quite stable for several months at a low water content.

Carob seeds need to have a pretreatment to stimulate imbibition prior to germination due to its natural dormancy.
The breaking of seed dormancy is usually obtained with a sulphuric acid treatment (Martins-Loução, 1986). Another possibility was to use the irradiation as a treatment in order to promote the breaking of carob seed dormancy as have also been reported earlier (Gunkel and Sparrow, 1967).

It was observed that radiation has a minimum effect on this (Fig. 2). Germination has been delayed in all group of seeds because they have had no sulphuric acid treatment. Control plants have been a reduction of almost 40% because they have no treatment at all. In all dose rates studied the lowest irradiation - 5,000 and 11,000 rad - promote the highest percentage of germination.

The pattern of the morphogenetic responses observed during carob seed are probably secondary effects of non-genetic physiological disturbances as have already been reported for other plant material after gamma irradiation (Gunkel and Sparrow, 1967).

It was observed that, treated seedlings have developed leaves though with some delay related to control (Fig. 3). Only the highest dose rate promote a strong growth inhibition.

Although high doses of ionizing radiation inevitably inhibit growth in plants, many reports have shown a stimulation by small exposures (Casarett, 1968). In our experiments it has been noted that there was an enhancement of different parameters observed in a high exposure of 22,000 rad. Under this dose rate, hypocotyl length (Fig. 4) cotyledon area (Fig. 5) suggest that a growth enhancement has been observed. This enhancement usually involves the early period of growth (Casarett, 1968). However in our experiments these seedlings have shown a great resistance to water stress, after high evaporation periods inside greenhouse.

Besides, shoot and internode growth (Fig. 6) are similar to normal control plants, after 6 months of germination.

The mechanisms involved are not clear to understand this phenomenon, specially when, in nature, the plant material can show a great genetic variability. There are some examples reporting dramatic increases in yield of various crops following exposure to ionizing radiation (Gunkel and Sparrow, 1968). An increase in dry weight of cotyledons (Fig. 7) both under seed exposures of 22,000 and 85,000 rad, has been observed. It seems possible that the metabolism of the seedlings might be sufficiently altered by a suitable dose of radiation to resultates an increase growth of cotyledons.
Fig. 1. Effect of radiation on germination of carob seeds.

Fig. 2. Effect of radiation as a treatment to promote the breaking of seed dormancy.

Fig. 3. Effect of radiation on leaves differentiation along carob seedlings development.

Fig. 4. Effect of radiation on hypocotyl length along carob seedlings development.
It does not seem inconsistent that a stimulating effect has been associated with the seed exposure of 22,000 rad since other growth enhancement have been observed (Figs. 4, 5, 6). However the increase in cotyledon dry weight associated with the increase of cotyledon thickness (Fig. 8) observed in seedlings with the highest dose rate does not mean a stimulating effect. On contrary, it is probably related with the strong inhibition of growth. Cotyledons have an important role during juvenile phase of carob tree plants (Martins-Loução, 1985). The presence of these nutrient rich and active organs during the initial phases of seedlings establishment could be considered as a physiological adaptation, permitting the plants to effectively establish themselves even under stress situations (Martins-Loução and Duarte, 1987). Apparently, the seedlings with the highest dose rate seem to channel into cotyledons all growth, instead of partitioning into root and leaves. These seedlings try to allocate in cotyledons all resources probably increasing total carbon gain, due to the high thickness (Fig. 8) and also nitrogen content (Fig. 9). Besides it is already known that generally plants have high nitrogen content under stress

Fig. 5. Effect of radiation on cotyledon area of carob seedlings during the development.

Fig. 6. Average of shoot and internode growth of carob plants after 6 months of germination. The transplant to pots was done after 2 months of germination.
The severe growth inhibition is also manifested by the development of chlorotic cotyledons (Fig. 10) with a complete alteration and reduction in photosynthesis (Fig. 11). Some reports show that photosynthesis is only slightly affected by radiation (Gunkel and Sparrow, 1967). In our experiments the ionizing radiation seem to have no effect on photosynthetic rate of cotyledons, instead a slight increase could be noted.

There are a considerable number of potential applications of ionizing radiation in food preservation. So far, very little actual commercial use has been made of these processes. From these experiments it was observed that the extent of adverse changes on growth depend on dose rate. In some cases the morphological changes were negligible or could be controlled after a recovery period (Fig. 6). The physiological changes are intended to be followed. Nevertheless it is important to note the great resistance of this seedlings that go on growing after 6 months. It seems reasonable to say that the application of this technique to carob storage may be a practical and useful fashion in the near future.

Fig. 7. Effect of radiation on cotyledon dry weight of carob seedlings during the development.

Fig. 8. Effect of radiation on cotyledon thickness - specific cotyledon weight - of carob seedlings during the development.
Fig. 9. Effect of radiation on nitrogen content of carob seedlings during the development.

Fig. 10. Effect of radiation on chlorophyll content of carob seedlings during the development.
Fig. 11. Effect of radiation on photosynthetic rate of carob seedlings during the development.


