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ORIGINAL PAPER

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Germination test for identification of irradiated garlic

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Abstract Ionising radiation can be used to inhibit the sprouting of garlic. This study investigates whether a simple germination test can be used to detect irradiated garlic. The results show that the germination test can be used as a detection method for garlic irradiated in the dormancy period. Detection is even reliable for samples treated with 25 Gy. For garlic irradiated after the dormancy period the germination test does not function properly. However, in this case the sprout-inhibiting effect of irradiation is also inadequate. For some samples, standard detection methods such as thermoluminescence, electron spin resonance and photostimulated luminescence were also applied. Whereas thermoluminescence measurements unequivocally proved the radiation treatment, electron spin resonance and photostimulated luminescence were not conclusive.

Keywords Garlic · Irradiation detection · Germination · Thermoluminescence · Electron spin resonance · Photostimulated luminescence

Introduction

Irradiation of bulb and tuber crops is mostly performed to inhibit sprouting [1, 2]. The irradiation of garlic is authorised in 23 countries worldwide (for example in Argentina, Belgium, Brazil, China, Egypt, France, India, Indonesia, Korea, Libya, Pakistan, Poland, Thailand; see the clearance database at http://www.iaea.org/icgfi, last updated April 2003). It is reported that up to 50,000 metric tons of garlic have been irradiated in China annually over the last few years [3]. The cleared maximum dose for garlic irradiation in most of countries is in the range of 40–200 Gy. Only a few countries accept higher maximum irradiation doses than this for garlic.

According to ICGFI Document 8 [4] and IAEA-TECDOC-937 [1], the irradiation dose for sprout inhibition of garlic depends on the time of irradiation after harvest. Shortly after harvest (1–2 months), when the bulbs are in the dormancy period, low doses, of 20–60 Gy, may be sufficient for 100% inhibition of sprouting. If irradiation takes place later than the dormancy period, higher doses, of 100–150 Gy, may be required. However, in this case, some sprouting occurs. The sprouts produced usually grow for a time, but later wither.

Irradiated garlic can be stored for 8–9 months at approximately 0 °C and 80–85% RH. At 25–33 °C, storage can be for only one month or less. At intermediate temperatures (10–11 °C) and 85–90% RH, storage may be for 6–7 months. Losses during storage are due to microbial spoilage and desiccation.

Bulb crops for fresh usage can also be stored untreated for sufficiently long duration at 0 °C, RH 65–70%. However, any deviations in temperature and humidity can lead to early sprouting.

In Europe, cold storage (below the freezing point) is recommended for bulbs, and before removal from storage they are thawed over a period of 1–2 weeks at about 4.4 °C. Spraying with maleic hydrazide 2–3 weeks before harvest is used in some countries, such as China and Brazil, to inhibit sprouting in onions and other bulb crops [1, 2]. This chemical treatment is not permitted in some countries (such as Germany and Romania).

Studies dealing with the detection of irradiated garlic apply analytical methods such as: tissue culture technique [5], electron spin resonance [6], thermoluminescence [7, 8], DNA comet assay [9] and measurement of 2-alkyl-

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cyclobutanones by GC-MS [10]. A recent paper described rooting of irradiated onions as a detection method [11].

In this study of the detection of irradiated garlic, a simple germination test has mainly been applied, but the thermoluminescence (TL) method (EN 1788) [12], the photostimulated luminescence (PSL) method (EN 13751) [13], and the electron spin resonance (ESR) method (EN 1787) [14] have also been applied.

Materials and methods

Garlic samples

Garlic samples used in this study were obtained from the Brazilian, German and Romanian markets. From the Brazilian market, five garlic samples (coded A, B, C, D and E) imported from China and suspected of being irradiated were tested. Every sample consisted of three bulbs of garlic. Four samples from the German market were tested. The garlic bulbs were purchased in Karlsruhe. They were produced by Öko-Garten, Duisburg (code 1), Ernteland, Ilsfeld (code 2), Malvi Carvati, Italy (code 3), unknown source (code 4). One sample supplied by a farm shortly after harvest was tested from the Romanian market. It should also be mentioned that it was not known whether the samples had been treated with any chemical intended to inhibit sprouting.

Irradiation and storage of samples

The samples from German (three bulbs per dose) and Romanian (five bulbs per dose) markets were irradiated at 25, 50, and 100 Gy. Irradiation was carried out at room temperature (20–25 °C) at the Nuclear Research Centre, Karlsruhe, Germany, in a Gammacell ^{60}Co source (Canada). The absorbed dose rate was 124 Gy/h. The doses were measured using Fricke solution [15]. The confidence level of dosimetry is ±5%. The garlic samples from the German market were treated late in the season, after the dormancy period. The Romanian garlic was treated shortly after harvest, while it was still in its dormancy period. After irradiation, the samples were stored for three months in the refrigerator (4 °C and 80% RH).

Detection methods

Germination test

Several cloves of garlic with the outer skin removed were placed on distilled water moistened filter paper in a covered Petri-dish and incubated at room temperature (20–25 °C) for several days. The test was similar to the procedure of Kawamura et al [16]. As can be noticed, the germination test is a very simple and cheap method. Its only inconvenience is the length of time (usual 4–5 days) until the results are read and evaluated. This method can be applied when time is not a problem.

Thermoluminescence method

TL measurements were carried out according to EN 1788 [12] using a Risó TL-DA-15 reader (Risó National Laboratory, Roskilde, Denmark). When enough silicate minerals (sand and dust particles) are isolated from a sample, this detection method is very reliable. It is a faster method (it takes about 2–3 days) than the germination test but needs work experience and is rather costly (TL reader, chemicals, irradiation source). This method can also be used to verify results obtained with other detection methods that are not specific for the radiation treatment.

Photostimulated luminescence method

PSL measurements were carried out according to EN 13751 [13] using a SURRC PPSL irradiated food screening system (SURRC, Glasgow, UK). This method is simple and very fast (about 15 min.), but the equipment is a bit expensive. When not enough minerals (sand and dust particles) are present at the surface of the sample this method probably does not work.

Electron spin resonance method

ESR measurements were carried out according to EN 1787 [14] using a Bruker EMS-104 EPR analyser (Bruker, Rheinstetten/Karlsruhe, Germany). This method is quite simple, radiation-specific and fast (15–60 min), but the ESR analyser is rather costly. Irradiated samples containing crystalline cellulose can be detected using this method.

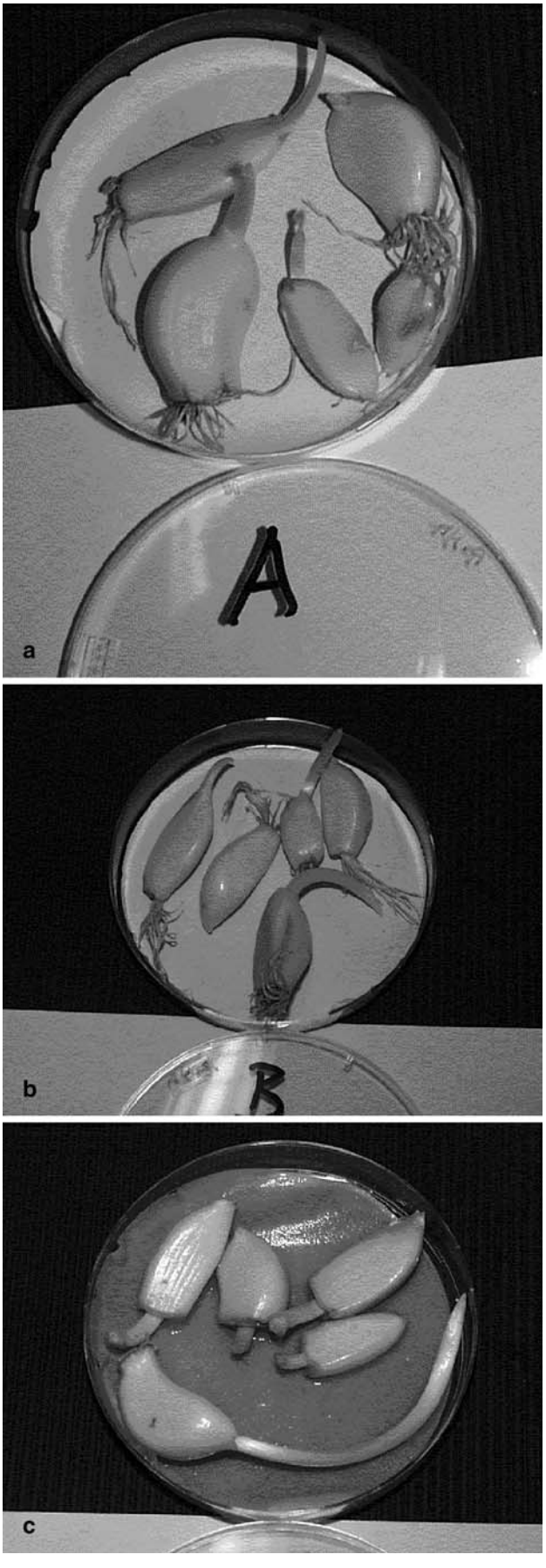
Results and discussion

Garlic samples from the Brazilian market

To these samples the germination test and measurements of TL and PSL were applied. The germination test showed some differences between the samples. Samples A, B, D, and E showed formation of multiple small roots and some longer shoots after 4 days of incubation. Sample C showed practically no root formation, but instead some long shoots appeared (see Fig. 1).

The TL measurements unequivocally demonstrated that sample C had been irradiated, whereas the samples A, B, D and E were non-irradiated. The Glow 1 curve of sample C clearly showed a maximum in the range 150–250 °C (Fig. 2), whereas the samples A, B, D, and E only emitted more light at higher traps, above 300 °C, which may result from low level radioactivity in the soil. After normalisation of the isolated minerals, using an irradiation dose of 100 Gy, the Glow 2 curve was clearly higher than 10×MDL and the TL ratio for sample C reached a value of 0.15 for the temperature interval I 200–258 °C, which was chosen according to Annex B in the EN 1788 [12]. The samples A, B, D, and E exhibited TL ratios of 0.084, 0.042, 0.040 and 0.027. Since the shape of the Glow 1 curve for sample C clearly indicated irradiation, this sample was identified unequivocally as having been treated by ionising radiation. It should be mentioned that the amount of silicate minerals from the garlic bulbs was very small (only three bulbs were available for each sample, therefore only 0.9–2.4 mg minerals were isolated). Nevertheless, detection was sensitive enough to detect the treatment status of the garlic.

The garlic samples were also measured according to the PSL method (EN 13751) [13], but the results were not conclusive. For all samples, values scattered around or below the lower threshold were obtained.



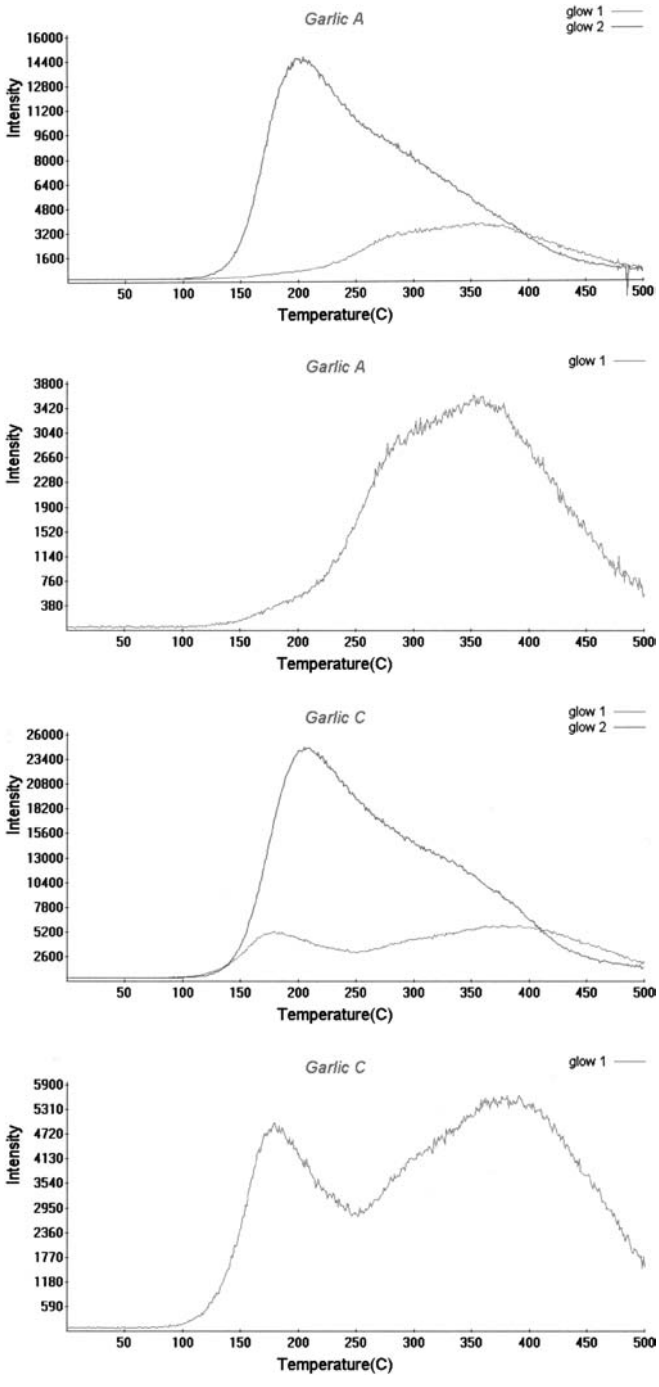


Fig. 2 Thermoluminescence glow curves of garlic samples A and C (for samples B, D, and E the TL glow curves look similar to that for sample A)

Fig. 1 Germination tests for garlic samples A, B, and C (samples D and E look similar to A and B)

Fig. 3 Germination tests for garlic samples (code 2) from the German market (samples with codes 1, 3 and 4 look similar)

Garlic samples from the German and Romanian markets

The garlic from the German market was treated 2–3 months later than the dormancy period (1–2 months after harvest). This may be why the samples developed both roots and shoots [2, 4]. Treated samples usually had shorter roots and longer shoots than the untreated ones (see Fig. 3). However, the differences between the samples did not allow a definite judgement about their irradiation status.

The results are quite similar to that for sample C of garlic from the Brazilian market. In that case there were very long shoots but no roots. For garlic from the German market the shoots were longer for treated samples than for untreated. The roots were shorter and sometimes there were no roots. The treated samples of garlic with code 3 developed roots for treated samples similar (in number and length) to those of the untreated ones.

The garlic sample from the Romanian market was treated in the dormancy period (shortly after harvest) and 100% inhibition of sprouting was expected. There was indeed no rooting or shooting even for a dose of 25 Gy (see Fig. 4). For this garlic, ESR measurements (EN 1787) [14] of bulb roots, bulb, and clove skins were also carried out, but the results were not conclusive. The irradiation dose used for sprout inhibition of garlic is probably too low to be manifested in the ESR spectrum.

Comparison of detection methods

The germination test proposed in this study is definitely the simplest and cheapest method. It remains to be seen, however, how chemical sprout inhibitors like maleic hydrazide influence germination. But, in any case, the germination test can be used as a screening test.

The culture technique for meristematic tissues from the garlic bulbs [5] is similar, but more complicated and users need some experience. It requires 1–2 weeks.

More rapid results would be obtained by measurement of 2-alkylcyclobutanones from the lipid part of the garlic. Due to the low level of lipids in garlic, sample preparation is cumbersome, and due to the low irradiation doses applied, the sensitivity of the standard assay (EN 1785) [17] must be increased. In principle, this has been achieved by N'Diaye [10], who made a fluorescent derivation of the 2-alkylcyclobutanones. The total effort, however, is very large, both in terms of instruments (GC-MS) and time involved (several days).

The DNA comet assay has also been successfully applied for the detection of irradiated garlic [9]. This method is rapid and simple, but users need some experience. The DNA comet assay should only be applied as a screening technique, since it is not radiation-specific (EN 13784) [18]. However, the use of maleic hydrazide on garlic did not cause DNA fragmentation, and chemically-treated samples could be discerned from irradiated samples [9].

The PSL method is also used, although mostly as a screening technique, since false positive and negative readings may occur. The PSL technique is very rapid but, in this study, was not conclusive for the irradiated garlic samples, possibly due to the low irradiation dose applied.

This statement also seems valid for ESR, since the results obtained were not conclusive. Unsatisfactory results when applying ESR to irradiated garlic have also been described by Desrosiers and McLaughlin [6].

The only method that could definitely prove the irradiation treatment of garlic in our study was the TL method. The successful application of TL to irradiated garlic has also been reported by Hwang et al [8] and Chung and Kwon [7], although the latter unfortunately did not apply a normalisation step. Although rather cumbersome and time-consuming (2–3 days), TL using the standard protocol EN 1788 [12] provides an unequivocal judgement about the irradiation status of garlic. Therefore, the simple germination test is recommended for a first screening and positive results should be subsequently confirmed by TL measurements.

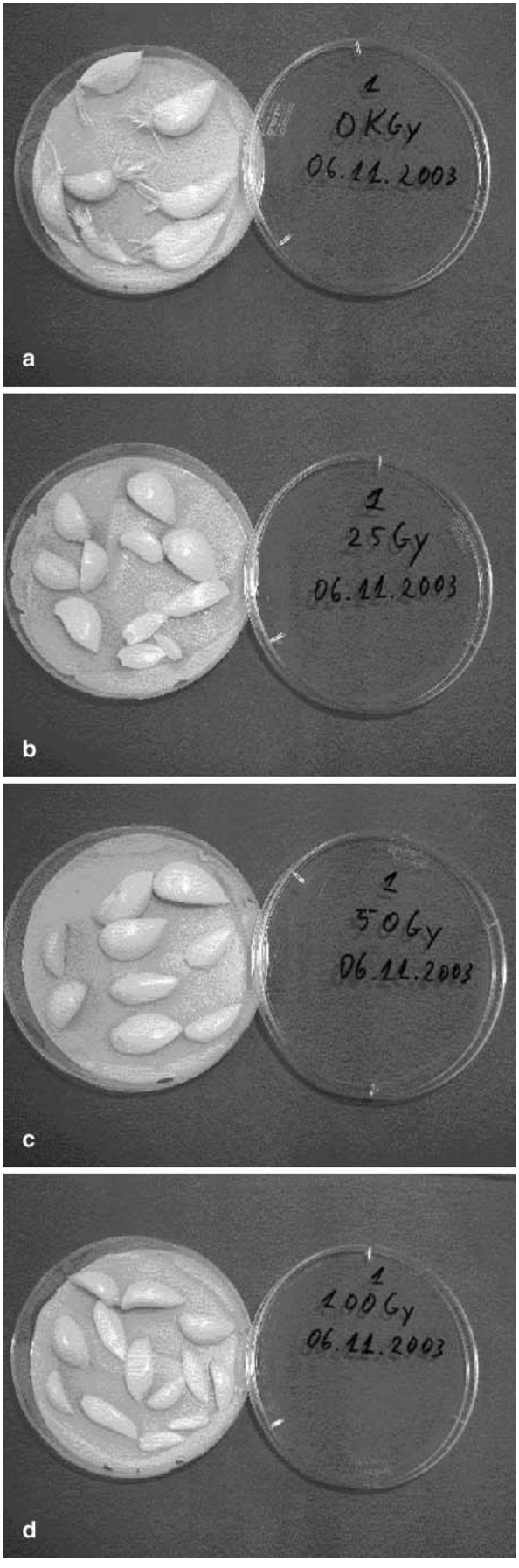


Fig. 4 Germination test for a garlic sample from the Romanian market

Conclusions

The irradiation of garlic for sprout inhibition is expected to be carried out in the dormancy period (1–2 months after harvest) because it is more effective (100% inhibition of sprouting) and cheaper (needs only 20–60 Gy). In this case, the germination test can give reliable results. The treated samples develop no roots or shoots. This is a very simple and inexpensive method and can be used as a screening method when there are many samples to be measured or when the test must be repeated often. The disadvantage of this method is the long period of time (4–5 days) the cloves need to germinate.

If the irradiation is carried out later than the dormancy period, shoots and roots can appear. What may be characteristic here is the fact that the roots are usually rather short and sometimes there are no roots. The shoots are also longer than usual. If after 4–5 days the test gives no clear results, germination could possibly be prolonged for 4–5 days more, in order to see if the sprouts wither.

The germination test can only be recommended as a screening method since it is not reliable enough to give a definite indication of the irradiation of a sample. Each positive result needs to be verified with a standardised detection method to specifically prove an irradiation treatment. For this purpose, the TL method is recommended.

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