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Determination of Threshold Concentration of Kanamycin to Transfer Gene in Cumin

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Research Article

Abstract

Cumin (*Cuminum cyminum* L.) is an annual plant belonging to the Apiaceae family is one of the oldest and economically most important medicinal spices. This study was conducted to determination of threshold kanamycin concentration in cumin for the purpose of gene transfer in a completely randomized design with three replications. Percentage of regenerated plants was recorded to study kanamycin effect. Results of analysis of variance under different concentrations of kanamycin indicated that there were significant differences between the concentrations at level 0.05. The results of mean comparison of different concentrations of kanamycin also indicated significant differences between them and categorized them in terms of their impact on growth and regeneration of cumin into four groups. The results of the experiment showed that the maximum regenerated plants were observed in the treatments of control, 50 and 100 mg/l kanamycin and the minimum regenerated plants were observed in concentrations of 250 and 300 mg/l kanamycin. Results demonstrated which in compared to other plants in cumin was required high concentration of kanamycin for selection. Generally, result suggested that kanamycin antibiotic can be used for selection of transgenic tissues of cumin in gene transfer programs.

Keywords: Cumin; Embryo; Kanamycin; Gene transfer.

1. Introduction

Cumin (*Cuminum cyminum* L.) is an annual plant belonging to the Apiaceae family, it is one of the oldest and most economically viable plants cultivated in warm regions such as Iran, India, Pakistan, Turkey, Egypt and Spain [1]. Historical evidence and recent studies report it as having pharmaceutical and medical importance [2]. Among spices cumin stands second to pepper with diverse applications [3]. However, cumin production has declined in recent years and the area of cumin cultivation is becoming depleted due to biotic stress such as fungal diseases. Fungal diseases such as fusarium wilt and root rot have been the most important cumin diseases in Iran and the world which have reduced grain yield and quality [4]. Conventional breeding methods have provided limited scopes to improve characters in cumin due to its low-genetic diversity and time-consuming approaches. Compared to other classical methods, gene transfer methods simultaneously provide the conditions of integration of several genes in a plant increasing the related resistance [5]. In gene transfer programs, selectable markers such as kanamycin usually are used to select transgenic tissues. Kanamycin binds to the active site of ribosomes of intracellular organelles and prevents protein synthesis and this leads to the degradation of chlorophyll and the occurrence of yellowness and whiteness in the plant and finally it's drying [6] . Kanamycin antibiotic has been used for selecting transgenic tissues of plants [7-9]. Up to now in cumin have been performed two experiments gene transfer which in them *hygromycin-phosphotransferase II* used as selectable markers [3,5]. No reports on determining the concentration of kanamycin threshold in cumin have been found yet. Therefore, present study was designed to measure kanamycin effect threshold in cumin for using in gene transfer programs.

2. Materials and methods

2.1 Embryos preparation and Pre-culture stage

Mature cumin seeds of a local ecotype (Kerman) were surface-sterilized and embryos were extracted from turgid seeds as described previously [3]. Embryo explants were prepared by removing a small part of the cotyledons and radicle from the

top and bottom of the embryo, respectively. Embryo explants as a pre-culture stage were first cultivated in the Murashig and Skoog (MS) medium containing sucrose (3% *W/V*) and plant growth regulators (6-benzyladenine (BA; 0.5µM) + α -naphthalene acetic acid (NAA; 2µM) for ten days [5].

2.2 Kanamycin concentrations and regeneration condition

After the pre-culture stage, small plants were transferred to the new medium which in addition to the above-mentioned compounds contained different kanamycin concentrations of 50, 100, 150, 200, 250 and 300 mg/l. Subculture on medium contained different kanamycin were performed every two weeks. The mediums were stored in a growth chamber at 16:8 h (light/dark) photoperiod at 24 ± 2°C with cool white fluorescent lamps of 35 µ mol m^{-2} s^{-1} light intensity for 6 weeks. After this period, percentage of mortality and regenerated plants in each Petri dish were recorded to study kanamycin effect.

2.3 Data analysis

The experiment was conducted in a completely randomized design with three replications. Five explants were culture in each Petri dish. Data were subjected to analysis of variance and Duncan's Multiple Range Test to evaluate the statistical significance among the means. The SAS and Excel software were used for data analysis.

3. Results and Discussion

Results of analysis of variance percentage of regenerated plants under different concentrations of kanamycin indicated that there were significant differences between the concentrations at level 0.05. (Table 1).

The results of mean comparison of different concentrations of kanamycin also indicated significant differences between them and categorized them in terms of their impact on growth and regeneration of cumin into four groups (a, b, c, d) (Figure 1).

Result showed that explants cultured on the control medium (free from kanamycin) naturally grew and produced mature plants (Figure 2). It should be mentioned that the cultured explants like the control treatment naturally grew and produced mature plants under 50 and 100 mg/l kanamycin concentrations (Figure 2).

The studies showed that fewer concentrations of kanamycin (about 50 mg) are used for the selection process in other plants [10,11]. Whereas the results of the study showed fewer kanamycin concentrations have not been effective for selecting cumin plants and the issue of explant escape will be occurred (Figure 1). This can be related to phenol compositions and

Table 1. Results of variance analysis of percentage of plants regenerated under different kanamycin concentrations.

Source of variance	Degree of freedom	Percent of regenerated plantlet
Concentration of Kanamycin	6	6838.5**
Error	14	19.05
Coefficient of Variation (%)	0.08	-

Note: * and **significant at the 0.05 and 0.01 level respectively.

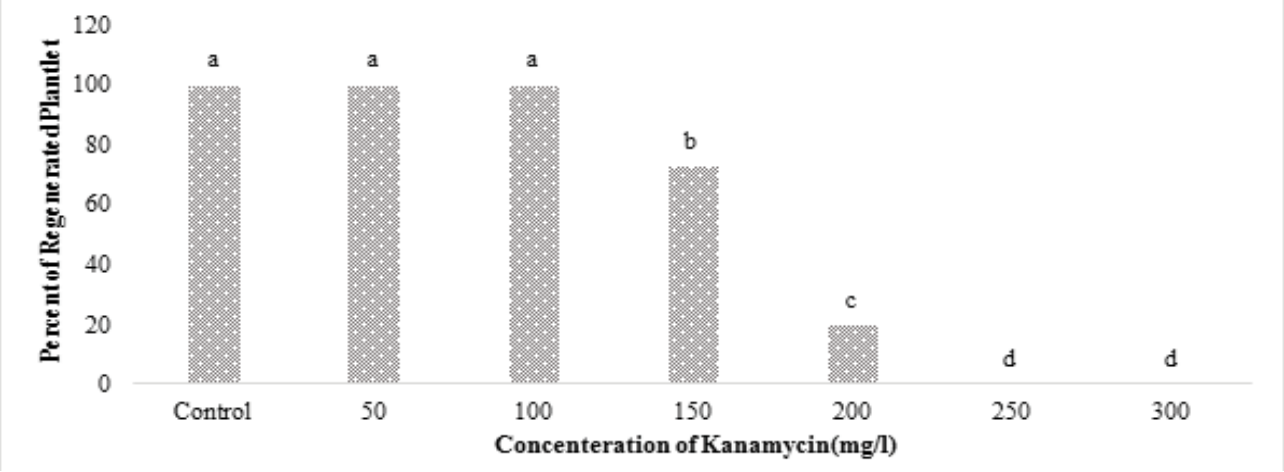

Figure 1. Mean comparisons of percentage of plants regenerated under different kanamycin concentrations using Duncan's method. The means with at least one common letter, do not have significant difference (P>0.05).

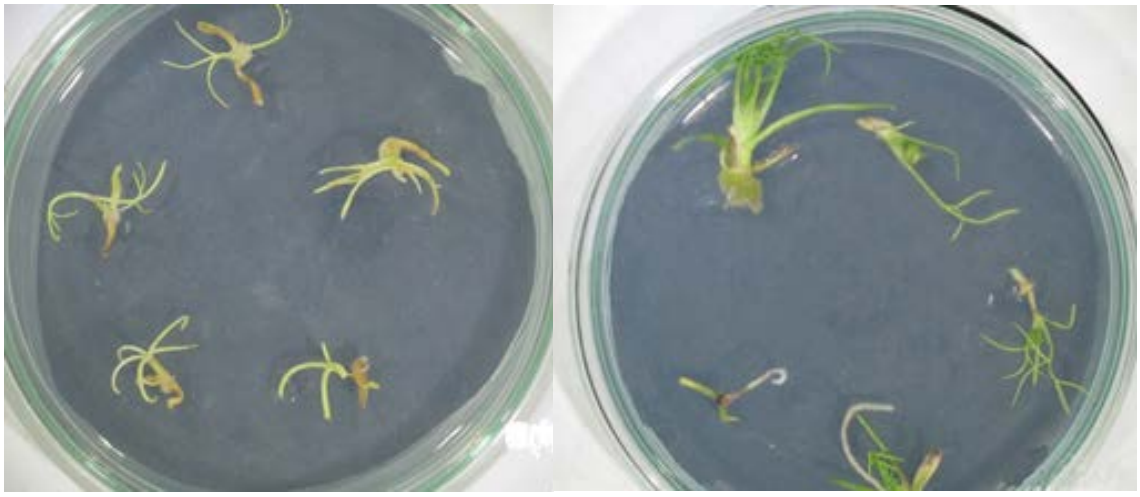

Figure 2. Plants derived from embryos on the medium lacking kanamycin (left) and containing 250 mg/l kanamycin (right).

secondary metabolites in cumin which have slowed down kanamycin destructive effect on plant cells and also caused resistance against low concentrations of antibiotics [12]. Baranski et al (2005) reported the desired concentration for carrot as a little more than the usual for other plants and nearly 100 mg [7]. The studies showed that after the passage of three weeks symptoms such as yellowness and burnt were observed in the cultivated explants in higher kanamycin concentrations including 150 and 200 mg/l. Results suggested that plants obtained from embryo explants under 250 and 300 mg/l kanamycin concentrations showed symptoms of yellowness, burnt, and growth cessation (Figure 2). The severity of the impact of the mentioned concentrations was in such a way that all the plants in them were completely turned yellow and get burnt. The results of the experiment showed that the maximum regenerated plants were observed in the treatments of control, 50 and 100 mg/l kanamycin and the minimum regenerated plants were observed in concentrations of 250 and 300 mg/l kanamycin. So regarding the results, kanamycin antibiotic can be used for selection of transgenic tissues of cumin in gene transfer programs.

4. Conclusion

The success of gene transfer programs and production of transgenic plants in cumin, besides having an effective regeneration system requires the selection of transgenic tissues using selector markers such as kanamycin. The results also showed that the issue of escape and resistance occurred in the explants. This phenomenon can be attributed to phenol compositions and secondary metabolites in cumin. Cumin biochemical compositions cause disorder in kanamycin destruction trend on plant cells so that symptoms of yellowness and burnt were not observed in explants under concentrations of 50 and 100 mg/l kanamycin as it was the case in the control treatment (without kanamycin). Results showed that as kanamycin concentration increased, number of regenerated plants decreased so that the concentration of 250 mg/l kanamycin caused symptoms of severe yellowness, burnt, and growth cessation in them. Results demonstrated which in compared to other plants in cumin was required high concentration of kanamycin for select transgenic tissues.

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