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An approach to enhance nutritive quality of groundnut (*Arachis hypogaea* L.) seed oil through endo mycorrhizal fertigation

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ABSTRACT

Groundnut is the sixth most important oilseed crop in the world and India is the second largest groundnut producing country. There is a need of increasing the production of groundnut and stabilizing its yield by using proper agricultural practices. Application of arbuscular mycorrhizal fungi (AMF) has been considered as an important strategy for sustainable agricultural practices. Approach of the present study was to evaluate effectiveness of ten different indigenous mycorrhizal species viz. *Glomus mosseae, Glomus clarum, Glomus fasciculatum, Glomus intraradices, Glomus ambisporum, Gigaspora gigantea, Acaulospora denticulata, Glomus globiferum, Gigaspora albida and Glomus pansiholus* on oil content, acid value, fatty acid profile and elemental status of groundnut oil. All the mycorrhizal treatments showed significant results as compared to the control (non mycorrhizal plants), but *Glomus mosseae* was found to be the most superior of all the ten mycorrhizal species. Oil percentage, extracted from *Glomus mosseae* (41.66%) treated groundnut oil was higher as compared to control (28.50%). Oleic acid, linoleic acid, palmitic acid content from groundnut oil of mycorrhiza treated plants varied in range of 36–45%, 16–22%, 13–18% respectively. Oil from *Glomus mosseae* treated groundnut plant showed increase in zinc, calcium, magnesium, manganese content than other mycorrhiza treated groundnut seed oil and control. Oil extracted from mycorrhiza treated groundnut oil showed decrease in acid value (AV) than control, which indicates higher stability of oil and longer shelf life. Therefore, it is very important to select the correct and specific species of AMF for improving the oil profile of Groundnut (*Arachis hypogaea* L.)

1. Introduction

Groundnut or peanut (*Arachis hypogaea* L.) is one of the important edible oilseed crop cultivated throughout the world. Groundnut crop is considered as an economically important crop in several countries of the world including India (Campos-Mondragón et al., 2009). Oil content of seed may vary depending upon variety, season and seed maturity. Poly Unsaturated Fatty Acid (PUFA) and Mono Unsaturated Fatty Acid (MUFA) are major components of groundnut oil (Jian et al., 2002; Jonnala et al., 2006). Groundnut oil contains more than ten fatty acids including palmitic acid (16:0) which approximately constitute 10%, oleic (18:0) and linoleic acid (18:2) together constitutes 80% of fatty acid composition in groundnut oil (Ahmed et al., 1982).

Groundnut seeds have high nutritional value, highly valued dietary constituents, proteins and phytochemicals such as phytosterols, phenolics, which have biological effects, like cardio protective, anti-inflammatory and anticancer effects (Venkatachalam et al., 2006). Groundnut oil comprises various micro and macronutrients like magnesium, zinc, manganese and calcium which provides considerable amounts of mineral elements to supplement the dietary requirements of humans and farm animals. Groundnut seeds oil contain approximately 9.5–19.0% total carbohydrates as both soluble and insoluble carbohydrates (Crocker et al., 1957; Rao et al., 1965; Oke, 1967; Abdel Rahman, 1982; Woodroof, 1983).

Usually, oil contains small quantity of free fatty acids along with the triglycerides. The free fatty acids content of oil is known as acid number or acid value. The storage duration of oil depends upon the free fatty acid content (Sadasivam et al., 2008). Free fatty acid composition of crop oil plays an important role in determining its functional properties, self-life, nutritional value and flavors of the food products derived from them (Lea, 1962).

Non-availability of seeds, poor soil fertility, and inappropriate crop management practices, pests and diseases are major factors responsible for poor yield of groundnut (Ahmed et al., 2010). As the consumer demands for high quality of PUFA and MUFA containing edible oils is increasing, there is need to increase groundnut production and its export to the world market.

Application of AMF has been considered as one of the important

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strategy for improvement of sustainable agricultural practices. It facilitates plant growth, increases nutritive quality of plant through enhancing uptake of several macro and micronutrients of low mobility from soil (Friese and Alle, 1991; Schüßler et al., 2001; Morgan et al., 2005). AMF, a group of plant symbionts, living in mutualistic association with the roots of most plant species, is involved in increasing the physiological growth, seed yield nutrient status, photosynthetic rates and nodule formation of the crop (Lambert et al., 1991; Augé, 2001; Garg et al., 2006). AMF also plays a vital role in enhancing biosynthesis of host beneficial phytochemicals, fatty acids and nutrient acquisition and positively induces changes in plant metabolism (Zeng et al., 2013).

Thus, the objective of the study was to evaluate the effect of ten indigenous mycorrhizal strains in improving the oil percentage, acid value, fatty acid profile, nutrient acquisition of *Arachis hypogaea* L.

2. Materials and methods

Groundnut seeds of Phule Pragati (JL-24) variety were collected from Agricultural Research Station, Mahatma Phule Krishi vidyapith, Jalgaon, India. Seeds with uniform size were selected for sowing to minimize errors in seed germination and were surface sterilized with sodium hypochlorite (0.5% w/v) before sowing. The seeds were sown along with ten different indigenous isolates of AM fungi *viz Glomus mosseae, Glomus clarum, Glomus fasciculatum, Glomus intraradices, Glomus ambisporum, Gigaspora gigantea, Acaulospora denticulata, Glomus globiferum, Gigaspora albida and Glomus pansiholus* and non mycorrhizal plants as control in green-house conditions, for the period of 120 days at 33 °C, with 65% relative humidity. Further, the harvested seeds of groundnut were processed for extraction of oil.

2.1. Oil Extraction

Groundnut *(Arachis hypogaea* L.) oil was extracted with n-hexane using Soxhlet method. Approximately 30 g of seeds were extracted with 400 ml n-hexane for 60–90 °C. After the extraction procedure, the solvent was evaporated under vacuum, and the extracted oil samples were subsequently stored at room temperature.

2.2. Oil content

The percentage of oil content was calculated as follows (Sadasivam and Manickam, 2008).

% of oil = Weight of oil obtained in gm/Weight of seed taken in gm × 100.

2.3. Acid value (AV)

The AV of groundnut oil was calculated using standard protocol of American Oil Chemists' Society (AOCS, 2009). Oil sample (5 g) was dissolved in toluene-isopropyl alcohol solution (1:1 v/v, 50 ml). Then, the solution was titrated with standard solution of potassium hydroxide in isopropyl alcohol (0.1 M), in the presence of phenolphthalein as the indicator. AV was calculated as

Acid value (mg KOH/g) = Titre value × 56.1 / Weight of the sample (g)

(56.1 = Molecular weight of KOH)

2.4. Preparation of Fatty acid methyl esters

The Fatty acid methyl ester (FAME) standards of methyl palmitate, methyl stearate, methyl oleate, methyl linoleate and methyl linolenate, were purchased from (Sigma Aldrich, India). Reagents used for methylation purpose (methanol, BF3–MeOH (14%), n-hexane, and sodium bicarbonate) were of analytical grade (TCI, India).

2.5. Boron- trifluoride methanol (BF_3-MeOH) methylation

For methyl esters preparation, 100 µl of Groundnut oil was mixed with 2 ml of boron tri fluoride in methanol solution (14%). The tubes were incubated at 55 °C for 1.5 h, with stirring the mixture after every 20 min. 2 ml of saturated sodium bicarbonate solution and 3 ml of n-hexane was added to the mixture. After proper mixing, the extracts were used for gas chromatography analysis (Jun et al., 2015; O'Fallon, 2007).

2.6. GC conditions for analysis of fatty acid profile

Fatty acid composition of the seed oil was determined using GC-2010 plus series Gas Chromatograph, (Shimazdu), equipped with the flame ionization detector and MEGA DetTBuSililBeta stainless steel packed column having internal diameter 0.25 mm, thickness 0.25 µm and length 30 m. The detector temperature was programmed for 200 °C, with a flow rate of 4 ml/min. The injector temperature was set at 280 °C and column temperature was programmed from 50 °C to 250 °C, with the increasing rate of temperature of 3 °C/min. Helium was used as the carrier gas. Peaks obtained were identified by calculating the retention time of samples with the standard analyzed at same conditions.

2.7. GC-MS analysis

The analysis was done using a QP-2010 Ultra Series gas chromatograph mass spectrometer,(Shimazdu), having a Rtx-5MS column (30 m × 0.25 mm; film thickness 0.25 µm) and DB 5973 mass selective detector. The injector and detector temperature were kept at 250 °C and 260 °C respectively. The oven temperature was programmed from 60 °C to 220 °C at the rate of 6 °C/min. Helium gas was employed as the carrier gas, at 1 ml/min flow rate; extracted oil samples were diluted with n-hexane and 0.1 µl was injected. The components of the oil were identified by comparison of their mass spectra with those of standard mass spectra from NIST Library (NIST 05).

2.8. Elemental analysis

The elemental composition of Groundnut seed soil was determined using Agilent 4100 Microwave Plasma Atomic Emission Spectrometer (MP-AES). Elements, mainly calcium, magnesium, manganese and zinc were determined from the extracted groundnut seed oil.

2.9. Statistical analysis

All the results were expressed as the mean ± standard deviation (SD). The data was analyzed by performing analysis of variance (ANOVA) and Duncan's multiple range test at $P < .05$ significance level using SPPS software, version 19.0 (IBM, New York, USA).

3. Results

Indigenous mycorrhizal isolates were inoculated individually to groundnut plant, to check whether mycorrhiza has effect on improving oil quality and nutritive profile. Hence oil extracted from mycorrhizal as well as control seeds were analyzed for oil yield, acid value, fatty acid profile and elemental status.

3.1. Oil content and acid value

A significant difference ($P < .05$) in oil content and acid value was observed in mycorrhiza treated groundnut seed oil than control. Oil content was significantly ($P < .05$) enhanced due to mycorrhizal inoculation. One and half fold increase was observed in *Glomus mosseae* (41.66 ± 0.73%) treated groundnut seed oil followed by *Glomus intraradices* (38.73 ± 0.73%) as compared to control (28.50 ± 0.54%).