Comparison of Resistance to Fusarium wilts disease in Seeded and Regenerated Sesame (*Sesamum indicum* L.)

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Abstract— *Plant tissue culture has been used as a tool for crop improvement in many different ways. Such as somaclonal variation that occurred in many different crops. In this study a program for disease resistance was established in sesame using somaclonal variation. As resistance to Fos is very important so different kinds and concentrations of Plant Growth Regulators were tested for producing of plantlet regenerated from apical shoot explants.*

The results showed that the combination of BA and NAA also BA and IAA with ABA could be used for regenerating sesame plantlets from apical shoots. The difference in BA concentrations had a positive effect on shoot and root regeneration and at least plant regeneration. So with combination of high level of BA and low level of NAA shooting from explants was dominant and with low level of BA and high level of NAA rooting was progressed. Regenerated plantlets and seeded planlets were compared for examining of resistance or susceptibility to Fos. The result showed that somaclonal variant resulted from regeneration of shoot and root of sesame could lead to producing resistant plantlets.

Keywords— fusarium, sesame, somaclonal variation.

Abbreviations: ABA **–** Abscisic acid; BA – 6 benzylaminopurin; *Fos – Fusarium oxysporum* fsp *sesami*, IAA – Indole-3 acetic acid; MS – Murashige and Skoog's medium 1962; NAA – α-naphthalene acetic acid; PGRs – Plant Growth Regulators.

I. INTRODUCTION

Somaclonal variation, resulting from a sum of genetic and epigenetic changes can induce mutations (Wei *et al* 2016). Somaclonal variation occurs through tissue culture in plants and plant tissue culture techniques proffer a substitute method of vegetative propagation of horticultural crops (Krishna *et al.* 2005; Alizadeh *et al*. 2010). On the other hand somaclonal variation is a basic method for inducing resistance in many plants against biotic and abiotic stresses (Chae *et al*., 1987; Kim *et al*., 1987 and Kariallappa, 2003). However Larkin and Scowkraft in 1981 coined a general term "somaclonal variation" for plant variants derived from any form of cell or tissue cultures, It is important that genetic variations occur in undifferentiated cells, isolated protoplasts, calli, tissues and morphological traits of in vitro raised plants (Bairu *et al.* 2011; Currais *et al*. 2013). So for inducing of variation in plants duo to somaclonal variation at first we need to a suitable and practical system for regeneration of plants. It means that denovo organs must to be regenerated by callusing phase. In this research we established a system for sesame regeneration and compared resistance of regenerated and seeded sesame against *Fusarium oxysporu*m fsp *sesami* that is a one of the most devastating agents for sesame in Iran. Also In Iran alike all over the world sesame is the quine of oilseed.

This important oilseed ranks third among the oilseed in production. Its oil content varies from 44 – 66 % containing two unsaturated fatty acids – oleic and linoleic together account for 85% (Maximum) with a combination of different essential amino acids and vitamins particularly β carotene (Brar, 1982 and Arslan *et al*., 2007).

II. MATERIALS AND METHODS

2.1 Preparation of plant materials

Seeds of sesame CV. Darab1 were obtained from Seed and Plant Improvement Institute, Karaj, Iran.

Mature seeds of sasame were aseptically sterilized by immersing in 100% commercial hypochlorite sodium (with 5% available chlorine) for 20, 30 and 60 min. Sterilized seeds then rinsed with sterilized distilled water for 4 – 5 times in order to remove the effects of disinfecting agent then cultured on 0.5 MS (Murashige and Skoog 1962), plus 3% sucrose and 0.7% agar in 9 cm petri dishes that were closed with parafilm. The cultures maintained in room temperature and mild light. Seven

days later pieces of hypocotyl (3 – 5 mm), apical shoots (2 – 3 mm) and cotyledon (9 – 16 mm^2) from seedlings were used as explants.

2.2 Media and culture conditions

For production of regenerated plants the explants were cultured on MS media supplemented with different kinds and concentrations of PGRs such as BA, IAA, NAA, ABA and 554.94 μm^{-1} myo-Inositol, 0.3 μml^{-1} Thiamin, 24.3 μml^{-1} Pyridoxine, 4.06 μml^{-1} Nicotinic acid , and 26.64 μml^{-1} Glycine. Explants were subcultured to fresh media with the same composition every 4 weeks duo to consumption of nutrition and oxygene. All media included 3% sucrose and 4% phytagel in this phase.

pH of all media was adjusted to 5.7 prior to autoclaving. All cultures were incubated at 25 ± 2 º C under a 16-h photoperiod and 1500 Lux illumination.

2.3 Inoculum preparation

To prepare of inoculum approximately 3 – 4 discs (1 cm^2 length) from PDA including *Fos* mycelia were cut and put on autoclaved wheat grains that placed in clear plastic bag. Clear plastic bag plugged by cotton and incubated at 25º for three weeks. Seventy five grams inoculum was mixed with two kg autoclaved soil and prepared for each pot before sowing.

Plantlets inoculation and evaluation of resistance

Two kinds of plantlets including seeded plantlets (4 foliage) and regenerated plantlets (approximately 4 foliage) planted in inoculated soil for evaluating for resistance or susceptibility against *Fos*. For evaluation of resistance dead or alive of plantlets was mentioned (See Pavlou)

III. RESULTS AND DISSCUTION

Explants (Leaf, hypocotyl and apical shoot) produced callus after nearly two weeks. Leaf explants doubled in size and produced callus on the wounded edges. Hypocotyl explants produced callus on their entire surface and apical shoots produced callus a little. The calli were apparently categorised based on the potential of organogenesis or embryogenesis. The effective factore on this classification was at first the component of media and the second explant type. So that on media supplemented with BA the adventitious shoots developed from hypocotyl and apical shoot within 14 – 28 days after callusing. Shoot developed much more by increasing of BA concentration. 4.44 and 22 μml^{-1} BA produced adventitious shoots with 5.70 μml^{-1} IAA + 3.78 $\mu ml^{-}1$ ABA and 1.61 μml^{-1} NAA respectively (Fig 1). Because a cluster of shoots usually formed on explants it was difficult to count the number of shoots on these two media. It is clear that regeneration efficiency in a range of BA (4.44 – 22 μml^{-1}) increased as BA concentrations increased but dense mass of shoots formed on explants affected not only by BA concentrations but also kind and concentration of auxins and other plant growth regulators. On the other hand BA concentrations with auxin(s) concentrations had influence in shoot regenerations. In Fig 7 the effect of BA and auxin(s) (NAA) on shoot regeneration was summerized. Also this picture shows that the kind of auxin(s) that cooperats with BA on shoot regeneration has an effective role. Shoots (1 – 2 cm) were excised and cultured on suitable rooting media especially medium with 8.05 μml^{-1} NAA and 0.13 μml^{-1} BA. After 7 - 10 days 5 – 6 white, thick and semi strong roots appeared on base of regenerated shoots (Fig 2). It is interesting that BA and NAA with different balances formed both shoot and root in sesame. Successful regeneration have a key role for somaclonal variation. At BA and NAA concentrations regeneration tended to shooting by rising in BA concentration and rooting by rinsing in NAA concentration. Similar results were observed in apple cultivars or rootstocks (Welanders, 1992; Ancherani *et al*., 1990; Yepes *et al*., 1994 and Famiani *et al*., 1994) where the low concentration of auxin in combination with height cytokinin content resulted in an increase in number of shoots per explants. Also there are many reports about the positive effect of BA in shooting (Lee *et al*., 2003; Ahmad *et al*., 2010; Rai *et al*., 2012; Kadota *et al*., 2001).

After establishment of regenerated plantlets and seeded plantlets the resistance (Fig 3 and Fig 4 respectively) of both compared against *Fos*. Two different kinds of plantlets displayed significant differences. According to Pavlou and Vakalounakis in 2005 sign of necrosis in xylem and phloem, dead or dying plantlets and wilt is indicator for susceptibility. As figures show four foliage seeded planlets after transplanting in inoculated soil displayed all of mentioned signs and dead after 20 days completely (Fig 5). So regenerated plantlets not only died but also grew and produced new leaflets after 12 – 20 days transplanting in inoculated soil (Fig 6). So we can result resistance to *Fos* altered in sesame by tissue culture. It is clear that a wide range of plant characteristics can be altered as a result of regeneration from cell and tissue culture including agronomacally important traits such as diseases resistance (Van den bulk 1991 and Sebastiani *et al*., 1994). Also Somaclonal

variation was claimed to be a source of variation for crop improvement (Ching – Yan Tang, 2005). Although somaclonal variation has been reported in crop with reproduction vegetatively (Larkin and Scowcroft, 1981) more there are a few reports about selection of somaclonal resistant to alternaria blight in sesame through tissue culture (Lokesha and Naik, 2011). On the other hand Fusarium is one of the most devastating microorganism that has been combatted by somaclonal variation (Pierk, 1994 and Ching Yan – Tang, 2005). Also this study that their results demonstrated the resistance of regenerated sesame plantlels against *Fos* agreed with researches that mentioned above. As figures show clearly one of the difficult in this research was weakness and smallness of regenerated plants when they compared with seeded plantlets duo to unfavourable conditions especially oxygen deficiency and high level of humidity. We tested seeded plantlets in 4 folige stage. Long-term maintenance (regenerated plantlets) in order to complete adaptation was not our purpose. But it is interesting that regenerated plantlets resisted against pathogen (*Fos*) despite of weakness and smallness.

So included in this study that would be presented method for inducing resistance in sesame by somaclonal variation as the one of results of tissue culture.

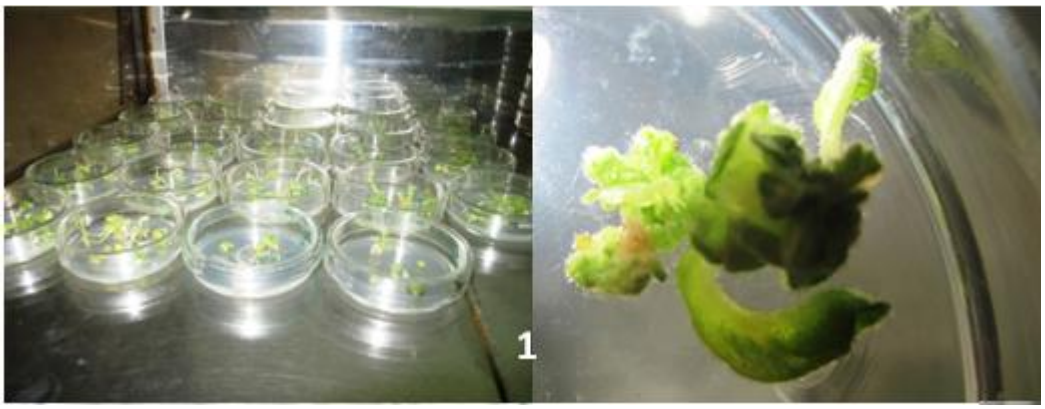

FIGURE 1 – 6. Occurance of somaclonal variation in regenerated sesame and the effect of it on resistance or susceptibility plantlets to *Fos*. 1- Shooting on apical shoots explants on MS medium with 8.88 µml^{-1} BA and 1.61 8.88 NAA. Rooting of regenerated shoots on MS medium with 8.05µml^{-1} NAA and 0.13 µml^{-1} BA. 3- Regenerated plantlets in pot. 4- Seeded plantlets in 4 foliage phase. 5-Dead seeded plantlets 20 days after inoculating with *Fos*. 6- Resistance of regenerated plantlets 20 days after inoculating with *Fos*.

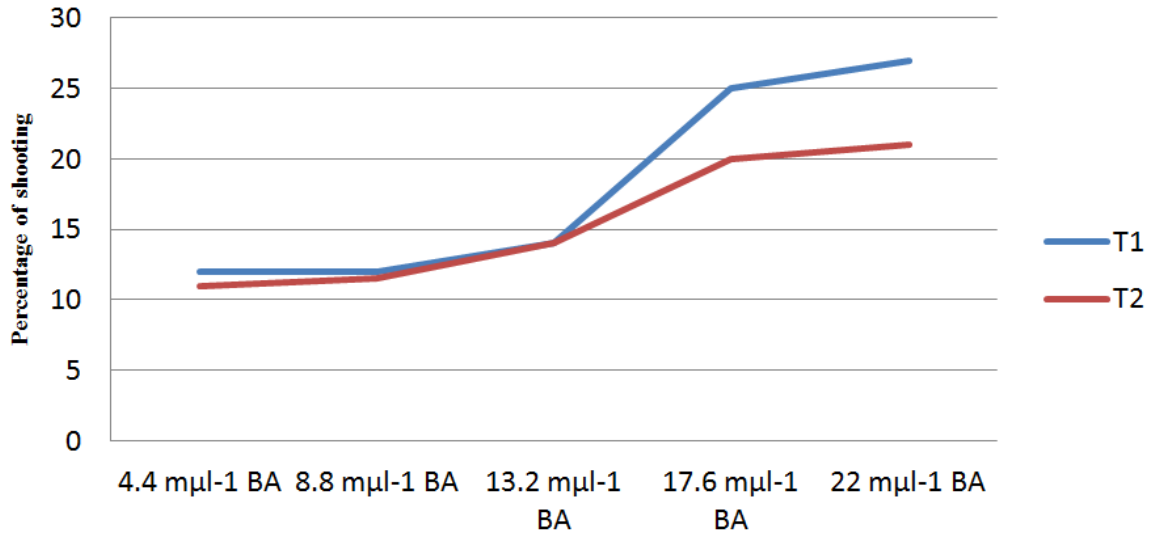

FIGURE 7. Effect of elavated BA concentrations with 5.7 mµl^{-1} IAA + 3.78 mµl^{-1} ABA (T1) and 1.61 mµl^{-1} NAA (T2)

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