Keywords: Castor, Induced systemic resistance. *Trichoderma*, Seedling blight

Seedling blight disease, caused by *Phytophthora parasitica* var *nicotiana*, is a major disease of castor (*Ricinus communis* L.) that results in yield reduction up to 30 to 77%. *Trichoderma*, a bio-control agent that can colonize root system of host plants is known to impart resistance against diseases through ISR as well as exert direct action against phyto-pathogens (Romera et al., 2019). Our studies examined the ISR in castor against seedling blight induced by different *Trichoderma* strains.

Seeds of DCS107, a susceptible genotype for seedling blight were treated with three strains of *T. asperellum viz*., TaDOR-N13, -TV5, -7316 and one strain, Th4D of T. harzianum. After the appearance of cotyledonary leaves, 10 day old discs of *Phytophthora parasitica* were placed on the abaxial side, of one of the two leaves. Leaf wetness, temperature (25°C) and humidity (about 70%) were maintained. Necrosis caused by the pathogen was measured at 48, 72 and 96 hours post infection (hpi) in three replicates each. In a modified experiment, a booster dose of *Trichoderma* suspension was given to the seedlings. To validate *Trichoderma*- mediated ISR within 24 hpi, total RNA isolated from the cotyledonary leaves (un-inoculated) of 12 day old castor seedlings treated with Th4d strain for 0, 1, 2, 3, 4, 6, 12, 16 and 24 h were subjected to RT-PCR and semi-quantitative RT-PCR using primers specific to PR1, PR2, PDF1.2a, OPR3 and Actingenes.

When compared with untreated seedlings, disease severity was reduced to 85.7% in Th4d treated seedlings. *Trichoderma* strains TaDOR7316 and N13 showed 50% and 42.9% of disease reduction over check (data not shown). In the 'booster' dose experiments, leaf blight size was considerably reduced when seedlings were given *Trichoderma* boost (Table 1). Th4d seed treated + 'booster' displayed better control of leaf blight (0.39cm), and N13 seed treated + 'booster' displayed infection diameter of 0.48cm when compared with control (1.53cm). RT-PCR analysis indicated up-regulation of the signature genes within two hpi, which continued until 24 hpi and by 48 hpi the expression levels started attenuating. These results indicated that ISR is initiated very early after interaction of *Trichoderma* with the castor roots. RT-PCR analysis with samples from the booster dose experiments, indicated increased expression after 'booster' dose of the *Trichoderma* on the roots of castor seedlings pre-treated with Trichoderma (seed treatment). The expression of the signature genes reached the maximum level within 6 hpi and maintained at that level even after 48 hpi indicating the prolonged ISR.

Table 1 Disease severity in different *Trichoderma* treatments

Treatments	Disease severity (%)	Reduction over check (%)
N13 seed treatment (ST)	47	41.25
N13 ST+ re-inoculation	14	82.5
Th4d ST	14	82.5
Th4d ST+ re-inoculation	5	93.75
TV5 ST	35	56.25
TV5 st+TV5 re-inoculation	60	25.00
7316 ST	40	50.00
7316 ST+ re-inoculation	40	50.00
No ST+Th4d re-inoculation	40	50.00
Check	80	0.00

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Robust and informative microsatellite markers for genetic improvement of Indian sesame (*Sesamum indicum* L.)

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ABSTRACT

Quality of sesame seed oil is superior but seed yields have stagnated at abysmally low levels. Marker-assisted genetic improvement holds key to break the yield-barrier. Robust molecular markers are the indispensable prerequisites of marker-assisted breeding. Microsatellites are the easy-to-use and codominant molecular markers widely used across crop species. In the present study, a set of 180 robust and informative SSR markers have been identified from about 400 SSR based primers tested and thus adding more markers for utilization in different marker assisted breeding programmes of sesame.

Keywords: SSR Markers, PCR optimization, Sesame, Molecular markers

Sesame (*Sesamum indicum* L.), is a nutritionally, economically, agronomically, and culturally important oilseed crop that is yet to be explored at molecular levels and in general, yield levels of the crop are almost stagnated for decades (Akhtar *et al*., 2009). Intervention with biotechnological tools such as molecular marker-assisted breeding is a significant modern approach to break yield barriers in sesame, as is accomplished in other field crops (Verma *et al*., 2019). Since the second-half of the previous decade genomic resources in sesame are increasingly becoming available (Dossa *et al*., 2017). Microsatellite markers are available in the public domain from various sources. However, most of them are not ready-to-use in Indian genotypes. Therefore, it is necessary to make them useful for genetic improvement of Indian sesame genotypes. With this motivation, the present study was conducted to develop robust and informative microsatellite markers for Indian sesame.

Publicly-available primer sequences were downloaded from various published works and databases (Dossa *et al*., 2017). Primers were synthesized and template DNA of Indian sesame genotypes were utilised. Two-dimensional gradient PCR, with respect to $MgCl_2$ concentration and annealing temperature, was performed on a thermocycler having gradient temperature facility. PCR-amplicons were run on 4% agarose electrophoresis and size-based alleles were ascertained.

Robustness and informativeness of microsatellite markers are determined by PCR parameters and allelic variations, respectively. Out of 400 microsatellite markers attempted, 180 markers showed consistent amplification in a panel of 50 morphologically diverse (data not given) Indian sesame genotypes with specifically optimized PCR parameters. Optimum annealing temperature raged from 55°C to 63°C and $MgCl_2$ concentration varied between 1.5 mM and 2.5 mM. A representative electropherogram of a set of 7 primers with one genotype is shown in Fig 1. Polymorphism-information-content (PIC) of 80 markers ranged between 0.32 and 0.65 with an average of 3.5 alleles per marker locus.

In conclusion, upon ensuring robustness by meticulously optimizing PCR parameters, microsatellites were made to reveal prevalent allelic spectrum of their respective loci in a given panel of Indian genotypes. A set of 180 robust and informative markers have been developed that could used in Indian sesame genetic improvement programs.

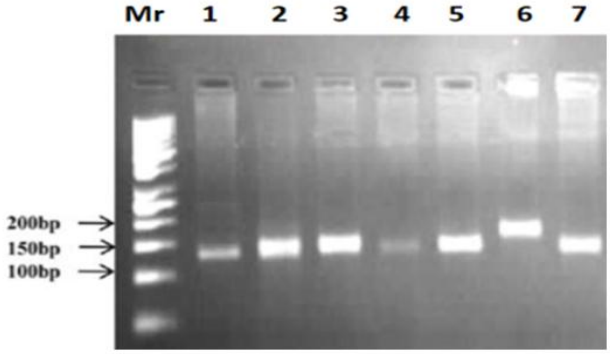

Fig. 1. Electropherogram showing amplicons of markers. Mr: marker; SSR markers: 1. SIM030, 2. SIM034, 3. SIM051, 4. SIM055, 5. SIM059, 6. SIM065, 7. SIM072, amplifying their respective loci in a IIOR sesame genotype NIC-16426-IS.

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Screening of new inbreds for their sterility and fertility reaction against new CMS lines in sunflower (*Helianthus annuus* L.)

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ABSTRACT

Sunflower, an important oilseed crop in the state of Andhra Pradesh witnessed a sharp decline in area during the recent past and the lack of diversity in CMS lines and inbreds is attributed as one of the major reasons. To address this issue, a set of new CMS lines (NDLA 2,3,4,5,6,7,8) and inbreds (CPI 1,6,8,10, NDI 1, 2, 12, 13, 14) of sunflower were tested. The results revealed that NDI 2 showed maintainer action whereas NDI 14, 15 segregated for fertility. CPI 1, 6, 8, 10 and NDI 1, 13 restored fertility. The identified restorers can be exploited either to strengthen the future hybrid breeding or in the synthesis of new restorers.

Keywords: CMS lines, Inbred lines, Fertility restoration, Sterility maintenance, Sunflower