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SHORT RESERECH ARTICLE

Inter-specific hybrids in *Areca* spp.: Verification using SCoT markers

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Abstract

Disease resistance has been a major goal in crop improvement programmes in arecanut (*Areca catechu* L.), where the fruit rot disease or *Mahali,* caused by *Phytophthora meadii*, is a major production constraint in India. The wild *Areca* spp., such as *A. triandra* and *A. concinna*, have been reported to possess resistance to *P. meadii.* Developing inter-specific hybrids between *A. catechu* and *A. triandra* or *A. concinna* could be one of the strategies to introgress the disease-resistant trait from the wild *Areca* spp. into cultivated arecanut. In this study, we report the utilization of start codon targeted (SCoT) markers to differentiate *A. catechu* from *A. triandra* and *A. concinna* and the development of sequence-characterized amplified region (SCAR) markers to enable authentication of true inter-specific hybrids between them. The technique would effectively verify inter-specific hybrids at the seedling stage itself.

Keywords: *Areca* spp., inter-specific hybrids, SCoT, SCAR

The arecanut palm, *Areca catechu* L., is the source of a common masticatory nut, commonly called areca nut or *supari*; its nuts are chewed regularly by at least 5% of the world population. India is the major producer and consumer of arecanut, sharing 62% of the area and 60% of the production; millions depend on arecanut crop for their livelihood. Many factors affect arecanut yield, with diseases being the major production constraint. Among the diseases, fruit rot, *Mahali*, or *Koleroga*, caused by the oomycete *Phytophthora meadii,* results in severe yield losses. The disease, which occurs in all arecanut-growing regions receiving heavy rainfall, can cause a 50 to 100% nut drop in palms if proper and timely control measures are not adopted. Bud rot and crown rot diseases can occur either as a further manifestation of the fruit rot infection or independently as fresh infection during the monsoon months or subsequent months (Saraswathy 2004).

The development of resistant varieties is an effective, economical and eco-friendly approach to manage plant diseases. Wild species/relatives of plants can play an important role in crop improvement programs as they are the storehouses of several important traits required for improving a particular crop. The genus *Areca* contains 76 species. *Areca triandra* Roxb. Ex Buch. -Ham., endemic to India, Bangladesh and other Southeast Asian regions, has a suckering habit compared to the cultivated species, *A. catechu*. The nuts are sometimes utilized as an inferior substitute for those of areca palm. *Areca concinna* Thwaites, a small clustering palm endemic to Sri Lanka, is similar to *A. triandra*- the difference between the two lies in the number of stamens. Challenge inoculation of *P. meadii* on intact leaf of embryo cultured plantlets of *A. triandra* and *A. concinna* and detached leaf segments from adult field-grown plants, keeping *A. catechu* as control, was carried out by Muralikrishna et al. (2018). Results revealed the resistance of *A. triandra* and *A. concinna* to *P. meadii* in intact and detached leaf segments, while the infection was severe in *A. catechu*. These results suggest that these

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resistant sources could be exploited to develop fruit rot disease-resistant arecanut hybrids through hybridization and biotechnological approaches. Subsequently, a crossing programme was chalked out, hybridization was carried out between *A. triandra* and the cultivated variety Mangala (*A. catechu*), and inter-specific hybrids were produced (ICAR-CPCRI 2018). Because of the perennial nature of the palms, it may take many years to verify the genetic purity of the hybrid.

The utility of the start codon targeted (SCoT; Collard and Mackill 2009) marker system in genetic diversity analysis in arecanut has been reported earlier (Rajesh et al. 2016a). The present investigation was carried out to utilize SCoT markers to differentiate *A. catechu* from *A. triandra* and *A. concinna* and to develop sequence-characterized amplified region (SCAR) markers to enable authentication of true inter-specific hybrids between them.

Spindle leaf samples of *A. catechu*, *A. concinna* and *A. triandra* were collected from ICAR-Central Plantation Crops Research Institute, Regional Station, Vittal. DNA was extracted by a modified SDS method (Rajesh et al. 2007). A total of 25 SCoT primers were used to amplify bulked DNA samples of five individual palms of each species as per the protocol of Rajesh et al. (2016 a, b). After amplification, the PCR products were separated on 1.2% agarose gel in 1X TBE buffer by electrophoresis and stained with ethidium bromide. The gels were visualized in a gel documentation system. Each reaction was repeated thrice to confirm the reliability of the results obtained. Primers capable of detecting polymorphism between *A. catechu* and its wild relatives were then validated in individual samples. SCoT22 primer could clearly differentiate *A. catechu* from the two wild species based on differences in the sizes of amplicons [around 900 bp in *A. catechu* and 1100 bp in *A. triandra* and *A. concinna*] (Fig. 1).

For the development of the SCAR marker, the unique amplicons obtained were excised directly from agarose gel, purified using a gel extraction kit (Qiagen), cloned (in pTZ57R/T; Fermentas) and sequenced. In *A. catechu,* we obtained an 892 bp length sequence from the SCoT 22 primer (NCBI Accession No. MK327520). Similarly, in *A. triandra*, a sequence of 1088p length was obtained from SCoT 22 (NCBI Accession No. MK327519). BLAST results showed that these sequences did not resemble any known nucleotide sequences. Based on these sequences, SCAR markers specific to *A. catechu* and *A. triandra* were designed (Table 1).

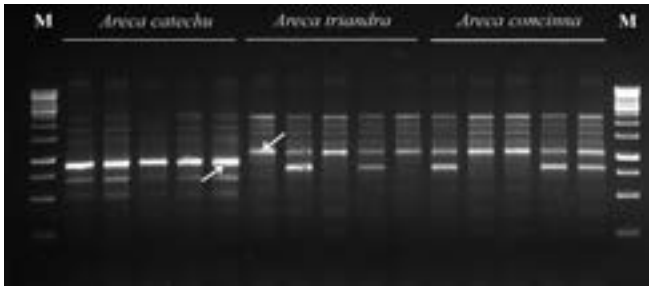

Fig. 1. SCoT banding patterns using SCoT 22. Arrowhead indicates polymorphic band

Validation of inter-specific hybrids using SCAR markers

The SCAR primers designed were validated in inter-specific hybrids between *Areca catechu* (Mangala cultivar) and *Areca triandra*. The combination of the two SCAR markers [SCAMNG22 and SCATRN22] gave a single amplicon in both parents, two parental bands in inter-specific hybrids and a single band in selfed progenies (*A. catechu*-specific band) (Fig. 2).

Recently, there has been a notable shift in the approach to plant genome analysis, moving away from traditional molecular markers to focus on gene-targeted ones like SCoT markers (Poczai et al. 2013). These molecular markers share technical similarities with commonly used dominant markers

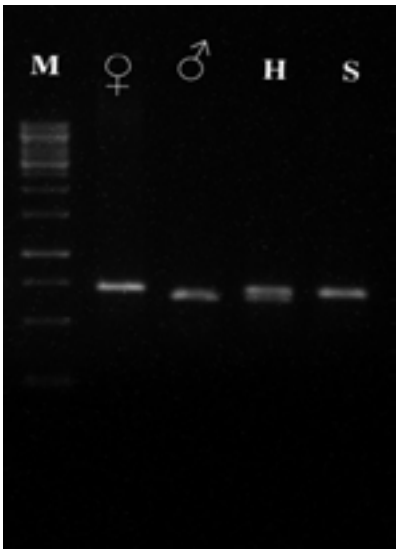

Fig. 2. PCR Amplification of parents and hybrids using a combination of the two SCAR markers [SCAMNG22 and SCATRN22]. M = 1 kb ladder; ♀ = female parent (*A. triandra*) ♂ = male parent (*A. catechu*); H = hybrid; S: selfed progeny

Table 1. Details of SCAR markers

Primer name	Forward primer (5′ 3′)	Reverse primer (5′ 3′)	Amplicon length (bp)
SCAMNG22	GTTGGCCCCAACGGTATG	CGATAGACTCCTCCACTTGGT	673
SCATRN22	TGTCAGCCAACTTTGCCTTT	TCCTCACCGCACTGACTATG	729

like RAPD or ISSR, utilizing a single primer amplification reaction without needing prior sequence or genomic information for primer design (Rai et al. 2023). These gene-targeted molecular markers are recognized as more effective and powerful because of their ability to target short-conserved gene sequences, enabling the detection of genetic variability within the genomic region associated with a beneficial trait (Rai et al. 2023). SCoT-derived SCAR markers have been developed to verify economically important Taxus media and related *Taxus* spp. (Hao et al. 2018) and closely related *Physalis* spp. (Feng et al. 2018). To conclude, the SCAR marker developed would be an invaluable tool to identify true inter-specific hybrids between arecanut and its wide relatives at the seedling stage itself.

Authors' contribution

Conceptualization of research (MKR); Designing of the experiments (MKR); Contribution of experimental materials (NRN); Execution of lab experiments (MKR, AAS, KSM); Analysis of data and interpretation (MKR, AAS, KSM); Preparation of the manuscript (MKR, NRN).

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References

Collard B. C. Y. and Mackill D. J. 2009. Start Codon Targeted (SCoT) Polymorphism: A simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Mol. Biol. Rep., **27**: 86-93.

Feng S., Zhu Y., Yu C., Jiao K., Jiang M., Lu J., Shen C., Ying Q. and Wang H. 2018. Development of species-specific SCAR markers, based on a SCoT analysis, to authenticate *Physalis* (Solanaceae) species. Front. Genet., **9**: 192. https://doi.org/10.3389/fgene.2018.00192.

Hao J., Jiao K., Yu C., Guo H., Zhu Y., Yang X., Zhang S., Zhang L., Feng S., Song Y. and Dong M. 2018. Development of SCoT-based SCAR marker for rapid authentication of *Taxus media*. Biochem. Genet., **56**: 255-266.

ICAR–CPCRI. 2018. Annual Report 2017-18. ICAR-Central Plantation Crops Research Institute Kasaragod, Kerala, India, pp. 196.

Muralikrishna K.S., Sharadraj K.M., Gangaraj K.P., Nagaraja N.R., Anitha Karun. and Rajesh M.K. 2018. *In vitro* assay for screening of *Areca* spp. for *Phytophthora* resistance. Int. J. Innov. Hortic., **7**(2): 139-142.

Rai M.K. 2023. Start codon targeted (SCoT) polymorphism marker in plant genome analysis: current status and prospects. Planta, **257**: 34. https://doi.org/10.1007/s00425-023-04067-6.

Rajesh M. K., Bharathi M. and Nagarajan P. 2007. Optimization of DNA isolation and RAPD technique in Arecanut (*Areca catechu* L.). Agrotropica, **19**: 31-34.

Rajesh M. K., Sabana A. A., Rachana K. E., Ananda K. S. and Karun A. 2016a. Potential of start codon targeted (SCoT) markers for assessment of genetic diversity of arecanut (*Areca catechu* L.). Indian J. Hortic., **73**(3): 423-6.

Rajesh M. K., Sabana A. A., Rachana K. E., Rahman S., Ananda K. S. and Karun A. 2016b. Development of a SCoT-derived SCAR marker associated with tall-type palm trait in arecanut and its utilization in hybrid (dwarf x tall) authentication. Indian J. Genet. Plant Breed., **76**(1): 119-122.

Saraswathy N. 2004. Diseases and disorders Arecanut. In: *Arecanut*, (ed. D. Balasimha & V. Rajagopal). Central Plantation Crops Research Institute, Kasaragod, Kerala: 131-189.