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SCoT polymorphism reveals genetic diversity in some important Fabaceae species

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ABSTRACT

Genetic variability of nine species of nutritional and medicinal important Fabaceae (faba bean (*Vicia faba),* fenugreek *(Trigonella foenum graecum),* chickpea *(Cicer arietinum),* lentil *(Lens culinaris),* soybean *(Glycine max),* lupine *(Lupines termis),* cowpea *(Vigna unguiculata),* bean *(Phaseollus vulgaris),* pea *(Pisum sativum)* were analyzed using start codon targeted (SCoT) marker system. DNA was extracted from the seedling of plants by DNeasy plant Minikit and 10 SCoT primers were used by PCR. One hundred and eighty three bands (183) were produced by SCoT markers generating (93.99) polymorphism. Dendogram analysis also reveals genetic variation among the nine species. The present study discloses the SCoT markers system as an effective technique for genetic diversity estimation of various leguminosae species. Such results boost utilization of the SCoT markers system in evolutionary studies, conservation and plant breeding.

Key words: Genetic variability, (SCoT) marker system, DNA

Introduction

The Fabaceae family comprises of 650 genera and more than18000 species (National Research Council 2002). The Fabaceae is considered one of the most significant sources of nourishment. Fabaceae are opulent in lysine and tryptophan but want to methionine and cysteine as well as the sulphur-containing amino acids. They are not expensive compared with the animal products, fish, and egg- consequently; they are eaten worldwide as main sources of protein and especially in the developing countries where feeding of animal protein may be limited (Beninger *et al.*1998; Mazur *et al* 1998). Different studies have indicated that they provide the diet with complex carbohydrates, soluble fibers, important vitamins, and metals, as well as polyphenols such as flavonoids, isoflavones, and lignin. Natural polyphenols employ their valuable health effects by their antioxidant activity. These compounds are able to remove free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals, and inhibit oxidases (Van Acker *et al.* 1998 and Alia *et al.,* 2003). It has been exposed that various parts of dry beans (hull and different fraction extracts) reveal antioxidant actions. For example; *Trigonella foenum-graecum* (fenugreek) seed extract has shown to have hypoglycaemic and hypocholesterolemic properties (Xue *et al* 2007). Also, *Vicia faba* (broad bean) displayed antimicrobial activity versus bacteria such as *Escherichia coli, Shigella sp*., *Bacillus subtilis* and *Staphylococcus aureus* (Peyvast and Khorsandi 2007) In addition, *Phaseolus vulgaris* contains a significant content of vitamin C (Jiratanan and, Liu 2004) as well as *vigna unguiculata* contains high content of phenolic compound and vitamin C (Oboh and Akindahunsi 2004; Oboh 2005)

Legumes create primary and secondary metabolites and other phytochemicals such as pharmaceuticals, nutraceuticals, pesticides, and industrial produces. Some of these plants were reported as medicinal plants, such as *Bauhinia kockiana*, *Bauhinia purpurea*, *Caesalpinia pulcherrima* and *Cassia surattensis* which have been employed conventionally to cure several diseases. Also, these medicinal plants revealed diverse bioactivities such as anti-cancer, anti-inflammatory, antimicrobial and antioxidant properties (Jo *et al* 2005). Phytochemicals are natural and non-nutritive bioactive compounds yielded by plants that act as protective agents against external stress and pathogenic attack (Chew *et al.* 2009).

Several novel marker techniques have been developed in proportion to the fast growing of genomic research (Gupta and Rustgi, 2004). Because of the great growth in open biological databases, the development of functional markers that are placed in or close to the candidate genes have become significantly simple (Andersen and Lubberstedt, 2003). Starting a direction other than random DNA markers to gene-targeted markers, an innovative marker system called StartCodon Targeted (SCoT) Polymorphism (Collard and Mackill, 2009) was established dependent on the short conserved region next to the ATG start codon in plant genes. Commonly SCoT markers are reproducible, however it is proposed

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that annealing temperature and primer length are not the only considerations controlling reproducibility. These are predominant markers identical to random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR). They may be benefitted for quantitative trait loci (QTL), genetic analysis, and bulk segregation analysis (Collard and Mackill, 2009). Basically, SCoT acts as RAPD and ISSR since the same single primer is utilized as the forward and reverse primer (Collard and Mackill, 2009; Gupta and Rustgi, 2004). Exploiting such markers in different analysis and diagnostic fingerprinting has been well verified in peanut, potato and grape (Gorji *et al.,* 2011; Guo *et al.,* 2012; Xiong *et al.,* 2011).

In the present study we aim to investigate the extent of genetic variety in nine plants of *Fabaceae* family using SCoT markers to renovate strategies for their preservation and sustainable exploitation.

Materials and Methods

1-Plant Material

The seeds of the nine used species was got from the agriculture research center and germinated to seedling with 10 cm tall. The young leaves were used to extraction of DNA.

2-Methods

DNA extraction and purification: DNA extraction from nine sample using a DNeasy Plant Mini Kit (Qiagen Santa Clarita, CA), this was performed following the manufacturer's instruction as follows: Grind plant tissue under liquid nitrogen to a fine powder using a mortar and pestle. Transfer the tissue powder and liquid nitrogen to an appropriately sized tube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw. A volume of 400 µl of buffer AP1 and 4 µl of RNase A stock solution (100mg / ml) were added to a maximum of 100 mg of ground plant tissue and vortexed vigorously. The mixture was incubated for 30 min at 65 ºC and mixed about 5 times during incubation by inverting the tube. Buffer AP2 130 µl was added to the lysate, mixed and incubated for 5 min on ice. The lysate was applied to the QIAshredder mini spin column, placed in a 2ml collection tube and centrifuged for 2 min at 20,000 xg (14.000 through fraction from step 4 was transferred to a new tube without disturbing the cell-debris pellet.1.5 volume of Buffer AP3/E was added to the cleared lysate and mixed by pipetting. A volume of 650 µl of the mixture from step 6, including any precipitate which may have formed, were applied to the DNeasy mini spin column sitting in a 2 ml collection tube. Centrifuged for 1 min at 6000 xg (8000 rpm) and flow-through was discarded. DNeasy mini spin column was placed in a new 2 ml collection tube, 500 µl buffer AW was added to the DNeasy mini spin column and centrifuged for 1 min at > 6000xg (>8000 rpm). Buffer AW 500 µl was added to the DNeasy mini spin column and centrifuged for 2 min at 20,000 xg (14,000 rpm) to dry the membrane. The DNeasy mini spin column was transferred to a 1.5 ml microcentrifuge tube and 100 µl of buffer AE was pipetted directly onto the DNeasy membrane. The microcentrifuge was incubated for 5 min at room temperature (15-25 ºC) and then centrifuged for 1 min at > 6000xg (>8000 rpm) to elute. Valuation for the DNA concentration: DNA concentration was determined by diluting the DNA 1:5 in dH_2O. The DNA samples were electrophoresed in 1% agarose gel against 10µg of a DNA size marker (Lambda DNA digested with HindIII and Phi x 174 DNA digested with HaeIII). Thus, estimation of the DNA concentration in a given sample was achieved by comparing the degree of fluorescence of the unknown DNA band with the different bands in the DNA size marker.

Start Codon Targeted (SCoT)

A) SCoT-PCR Reactions

SCot assay was achieved as described in (Collard and Mackill, 2009) for nine samples using ten primers (Table 1). The PCR amplification was done in a total volume of 25ul, containing 1X reaction buffer (10 mM Tris-HCl, pH 8.3 and 50 mM KCl), 1.5 mM MgCl2, 1U Taq DNA polymerase (promega), 2.5mM dNTPs, 25 poml of primer and 30 ng genomic DNA.

Table 1: Sequences of the ten primers used in SCoT.

Primer Name	Sequence (5'-3')
SCoT 7	ACAATGGCTACCACTGAC
SCoT 9	ACAATGGCTACCACTGCC
SCoT 10	ACAATGGCTACCACCAGC
SCoT 11	ACAATGGCTACCACTACC
SCoT 14	ACCATGGCTACCAGCGCG
SCoT 24	CCATGGCTACCACCGCAG
SCoT 28	CAACAATGGCTACCACCA
SCoT 32	CAACAATGGCTACCACGC
SCoT 35	AACCATGGCTACCACCAC
SCoT 46	ACCATGGCTACCACCGCC

B) SCoT- thermocycling profile and Detection of Products

PCR amplification was carried out in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) thermocycler. The SCoT amplification conditions were as follows: an initial extended step of denaturation at 94°C for 5 min followed by 40 cycles of denaturation at 94°C for 1 min, primer annealing at 50°C for 1 min and elongation at 72°C for 1.5 min. The primer extension segment was extended to 7 min at 72°C in the final cycle. The amplification products were resolved by electrophoresis on 1.5% agarose gel containing ethidium bromide (0.5 ng/ml) in 1X TBE buffer. A 100 bp DNA plus ladder was used as a molecular size standard. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD)

Analyses of the banding patterns generated by SCoT markers were compared to govern the genetic relatedness among the 9 plant species. Clear and distinct amplification products were recorded as '1' for the presence and '0' for the absence. The similarity matrix was used in the cluster analysis. This method is called Unweighted Pair Group Method using Arithmetic Average (UPGMA) (Sneath and Sokal, 1973).

Results

The 10 SCoT primers created an overall band profile of 183 fragments with an average 18.3 fragment/primer. The product size varied between 200 and 1850 bp (Table 2 and Fig. 1). The maximum number of bands (23) is at SCoT-11, while the minimum number is 12 bands at SCoT-32. The highest polymorphism percentage (100%) is at scot11, 14 and 35 while the lowest percentage 86% at SCot32.The monomorphic bands are 11 bands with an average 1.1 fragments/primer. The maximum number of monomorphic bands is 2 bands at SCot 24, 28, 32 and 46 and minimum number is at SCot 16.

A dendrogram has been generated using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) with sequential agglomerative hierarchical and nested clustering method program in NTSYS (SAHN) to display a phenetic representation of genetic relationships as revealed by similarity coefficient. Low similarity means low relationship and high similarity indicates high relationship. The dendogram Fig. 3 is divided into 2 clusters. The first one is separated into 2 sub-clusters, each contains 2 species, (*vigina unguiculata* and *lens culinaris)* in the first and (*cicer arietinum* and *trigonela foenum graecum)* in the second sub-cluster. The second cluster is divided into 3 sub-clusters. One of them contains (*lupines termis* and *pisum sativum)*, the second contains (*vicia faba* and *glycine max*) and the third contains only *phaseollus vulgaris.*

Table 2: 10 SCoT Primers used to genotype of the nine legume plants

Primer name	Primer Sequence (5'-3')	Mono mophic	Poly morphic	unique	Poly morphic with uniq	Total no. of bands	Poly morphism	Mean of Bands frequency
SCoT 7	ACAATGGCTACCACTGAC	1	15	3	18	19	95%	0.4
SCoT 9	ACAATGGCTACCACTGCC	1	15	3	18	19	93%	0.54
SCoT10	ACAATGGCTACCACCAGC	1	11	10	21	22	92%	0.36
SCoT 11	ACAATGGCTACCACTACC	0	19	4	23	23	100%	0.36
SCoT 14	ACCATGGCTACCAGCGCG	0	17	3	20	20	100%	0.37
SCoT24	CCATGGCTACCACCGCAG	2	17	2	19	21	90%	0.45
SCoT 28	CAACAATGGCTACCACCA	2	13	0	13	15	87%	0.44
SCoT 32	CAACAATGGCTACCACGC	2	9	3	12	14	86%	0.44
SCoT 35	AACCATGGCTACCACCAC	0	14	1	15	15	100%	0.5
SCoT 46	ACCATGGCTACCACCGCC	2	13	0	13	15	87%	0.56
	Total	11	143	29	172	183	93.995	

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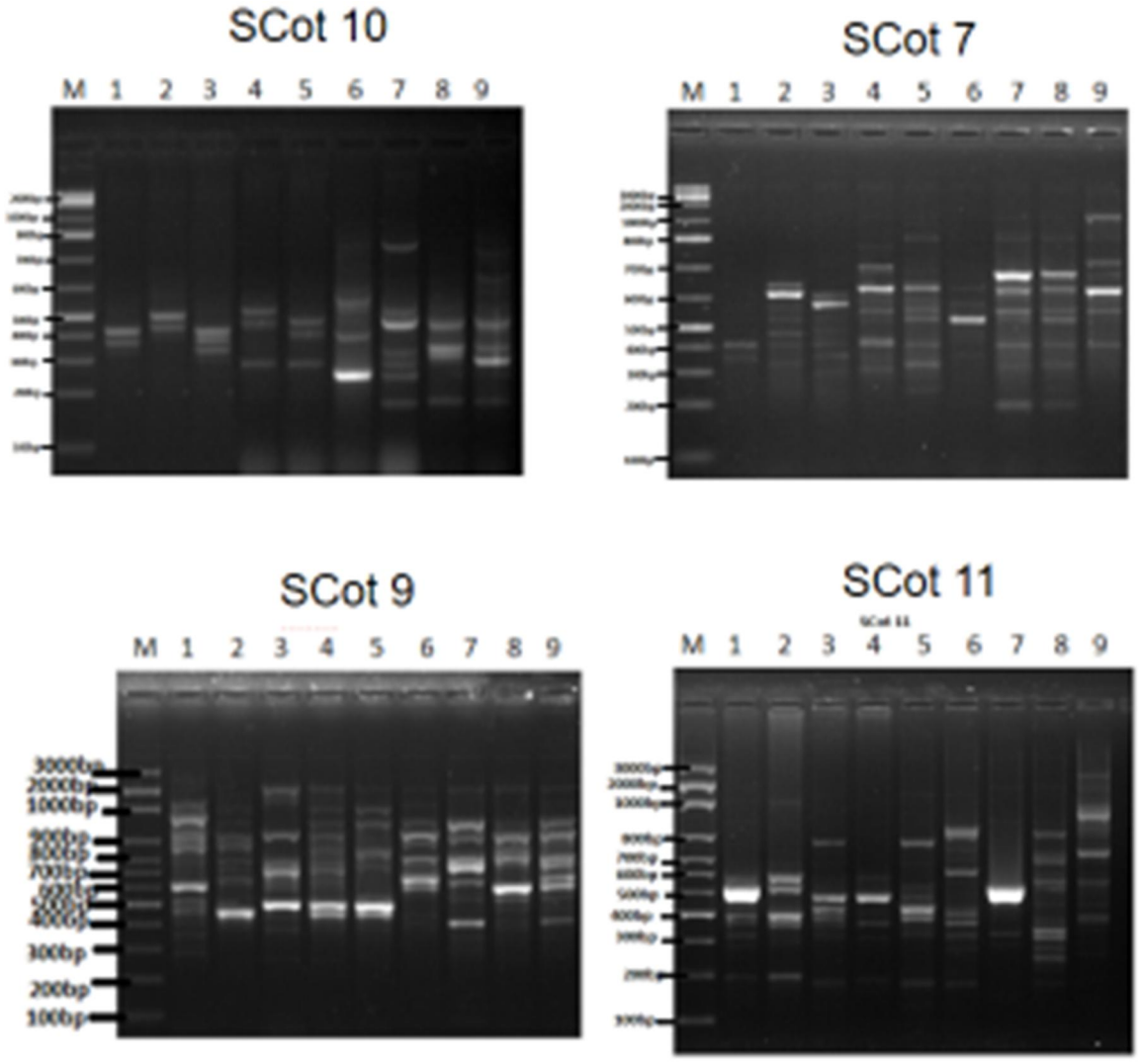

Fig. 1: Banding profile in 1- *vicia faba* 2- *phaseolus vulgareis* 3-*glycine max* 4 *lupinus termis* 5- *pisum sativum* 6-*vigna unguiculata* 7- *cicer arietinum* 8- *lens culinaris* 9-*trigonela foenum graecum* using SCot primers (7,9,10,11).

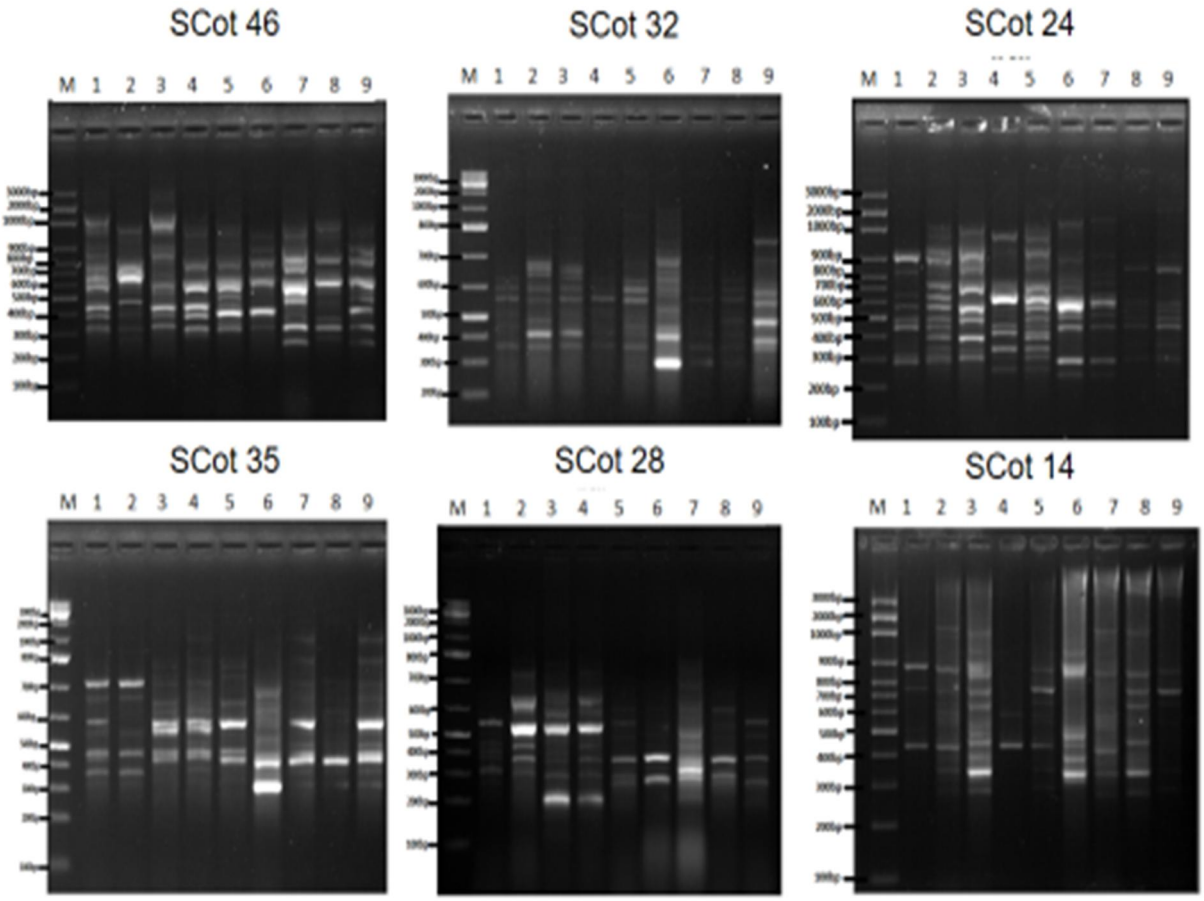

Fig. 2: Banding profile in 1- *vicia faba* 2- *phaseolus vulgareis* 3- *glycine max* 4- *lupinus termis* 5- *pisum sativum* 6-*vigna unguiculata* 7- *cicer arietinum* 8- *lens culinaris* 9-*trigonela foenum graecum* using SCot primers(14,24,28,32,35,46).

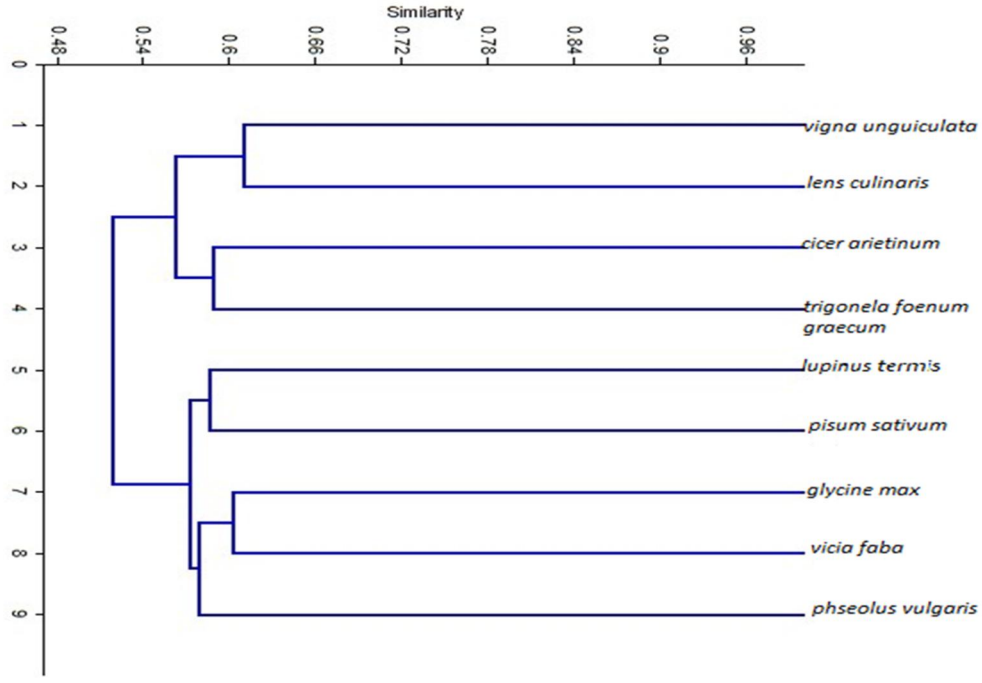

Fig. 3: Dendogram of 9 legume species constructed from the SCoT using UPGMA and similarity matrics computed according to Dice coefficient

Discussion

Different tools were used to determine the genetic relation between members of *fabaceae* as morphological marker and protein electrophoresis. But the relation at some plants need additional techniques such as molecular markers like random amplified polymorphic DNA (RAPD) (Alege *et al.,* 2014) and SCoT primers. SCoT markers were used in this study due to a number of advantages to other marker techniques. It provides easier development of species-specific primers than SSR (Jiang, *et al.,* 2014), lower cost than AFLP (Jiang, *et al.,* 2014) and higher reproducibility than RAPD (Xiong *et al.,* 2011). In some cases it is recommended that molecular markers such as SCoT should be employed to compliment the findings of protein electrophoresis study (Alege *et al.,* 2014)

From the dendogram Fig. 3 the *phaseolus vulgare* is found alone in one group it is agree with legume protein genetic diversity (Valizadeh, 2001). The relation between *phaseolus vulgare* and *cicer arietinum* is the same as (Amal and Mondal, (2010). The polymorphism among the nine plants is in harmony with (Valizadeh, 2001). The dendogram relation among *phaseolus vulgare; vigna unguiculata and glycine max* agree with legume seed protein electrophoresis (Alege *et al.,* 2014). Also, the present results are supported by the rbcL data (Doyle *et al.,* 2000 and Martin Wojciechowski *et al.,* 2004). Therefore, the results of the current study show that, SCoT markers are efficient in assessing the genetic diversity among *fabaceae.*

Conclusion

In the present study, we used 10 SCoT primers to evaluate the nine fabaceae species diversity. This is the first application of SCoT marker for some species of the *fabaceae* family that have nutritional and medicinal importance. Considerable number (93.99) of polymorphism among species was produced in this study. This significant number of polymorphic bands indicates the powerful of SCoT marker as a fingerprinting and diversity analyzer. Consequently, the SCoT marker could possess the ability to characterize various other species belonging to the family of *fabaceae* which ultimately would be helpful in plant breeding applications. Evidence of the amount and spreading of genetic variation as well as relationships among breeding materials play an important role in crop enhancement.

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