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Research Article

Identification of contrasting genotypes for Fusarium wilt disease in *Mucuna pruriens* germplasm through combined *in vitro* screening and AFLP analysis

Mahesh, S and Sathyanarayana, N*
Department of Biotechnology, Sir M Visvesvaraya Institute of Technology, Yelahanka, Bangalore – 562 157
*E-mail: sathya_dixit@yahoo.com

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Abstract:
Fifteen germplasm accessions identified on earlier field data were screened for Fusarium wilt resistance using *in vitro* culture filtrate approach. Infection and disease assessment were carried out based on wilt incidence and percentage mortality of the plants. Overall screening results revealed that 5 out of 15 accessions were moderate to highly resistant while 6 accessions were highly susceptible to Fusarium wilt. The results were further supported by differences in root and shoot weight and length amongst susceptible and resistant genotypes. Polymorphism analysis based on AFLP data revealed only a sub-set of these divergent genotypes confirming to genetic divergence levels required for raising segregant population. Based on the results of combined analyses, the genotypes 500108KA and IC471870, 500101KA and 500155AP have been recommended as suitable parents for developing population for mapping Fusarium wilt resistance in Velvetbean.

Key words
Mucuna pruriens var. *utilis,* Fusarium wilt, disease screening, AFLP, contrasting genotypes

Introduction

Mucuna pruriens (Velvetbean- Fabaceae) has gained increased global attention in recent years as promising source of protein diet due to presence of 20-30% of protein content in the seeds (Buckles 1995). It is also a prominent source of L-Dopa (L-3, 4 dihydroxyphenylalanine) – a non protein amino acid that acts as a precursor of the neurotransmitter drug dopamine used in the treatment of Parkinson's disease (Haq 1983). Although the plant exhibits wide ranging resistance (Rich *et al.,* 2003) which is mainly attributed to accumulation of high L-Dopa content in different plant parts (Bell and Janzen, 1971), there are few reports of damage caused by different pests and diseases (Berner *et al.,* 1992; Keinath et al., 2003; Sridhar and Rani, 2004). During our field evaluation from 2007-2010, extensive damage to germplasm accessions, especially among the cultivated group, was noticed due to Fusarium wilt disease caused by *Fusarium oxysporum*. Despite a familiar wilt causing pathogen in other legume crops (Kraft, 1994; Infantino *et al.,* 1996), it was never reported earlier in Velvetbean. Being a soil borne disease it cannot be effectively managed through fungicides, and hence screening for source of genetic resistance has become imperative.

Broad range of approaches has been employed earlier to screen disease resistance among germplasm collections (Trigiano *et al.,* 2004). *In vitro* screening has been one of the most efficient methods and represents an immediate and inexpensive way of selecting plant variants with tolerance to either the pathogen or its toxin, as compared to classical methods (Svábova and Lebeda, 2005). It has found wide application in screening resistance in several crop species like *Pisum sativum,* Alfalfa and soyabean (Lebeda and Svabova, 1997; Cvikrova *et al.,* 1992; Huang and Hartman, 1998). Such efforts, along with, others have allowed introgressing desired traits to economically important varieties without dramatically changing other desirable agronomical and resistance characters (Evans and Sharp, 1986).

Utility of DNA markers for genetic polymorphism studies and selection of divergent parents is widely reported (Kumar, 1999; Rommens and Kishore, 2000; Venkatachalam *et al.,* 2008). Among the different marker systems available at present, AFLP represents one of the most reliable techniques as it combines assay flexibility with high degree of sensitivity and reproducibility (Vos *et al.,* 1995). It is also extremely proficient in revealing diversity at the genetic level and provides an effective means of covering a wide area of the genome in a single assay (Karp and Edwards, 1997). Consequently AFLP has found extensive applications in developing markers associated with disease resistance in *Triticum* (Najimi

et al., 2002), *Hordeum* (Altinkut *et al.,* 2003), *Lycopersicon* (Giovanni *et al.,* 2004), *Malus* (James *et al.,* 2004) and *Oryza* (Jain *et al.,* 2004). In the present study we have analyzed in conjunction, the disease screening data with genetic polymorphism information obtained through AFLP studies to select genotypes contrasting for wilt disease that can be used to map the genomic loci responsible for Fusarium wilt disease in Velvetbean.

Material and Methods

The plant material: Fifteen *M. pruriens* var. *utilis* accessions identified earlier during preliminary field evaluation programs carried out at Sir M. Visvesvaraya Institute of Technology, Bangalore were used in the present study. Among the selected genotypes, eight accessions collected from diverse agro-climatic regions in India and the seven obtained from repository of National Bureau of Plant Genetic Resources (NBPGR), New Delhi (Table 1). The seeds recovered from the healthy pods were used in all the experiments.

Isolation and Identification of Fungi:The isolation and identification of fungal pathogen was carried out as per the method described by Kim *et al.* (2005). The damaged tissues from the stem and root regions of the infected field- grown plants were cut into 5 mm length and were placed on Czapek Dox agar (CDA) after surface sterilization with 70% ethanol for 1 min. The plates were then incubated at 25^0C. After five days, the mycelial tips of the fungal isolates were cut and transferred to fresh medium. Culture characteristics of the isolates were recorded at regular intervals and morphological characteristics of microconidia were examined after 10 days of incubation. The identification *of F. oxysporum* was confirmed by comparing the morphological characters of the pathogen with the keys of Booth (1970) and Nelson *et al.* (1983).

Inoculum preparation : Aberkane *et al.* (2002) protocol was adopted with modification for preparation of inoculum. The pathogen isolates from the pure culture were sub-cultured on Czapek Dox agar slants and incubated at 35°C. Inoculum suspensions were prepared from mature 7-days old cultures by suspending the conidia in 5 ml of sterile distilled water. To get optimum suspension, the conidia from the colonies were rubbed carefully with sterile cotton swab, re-suspended and homogenized for 15 s in a vortex mixer. Appropriate dilutions were later performed to adjust the final inoculum concentration to 1.0 x 10^6 spores/ml by microscopic enumeration using haemocytometer.

Germination of Velvetbean seeds: Mature seeds collected from well-dried pods of *M. pruriens* var. *utilis* were used as the seed source. Prior to inoculation, the seeds were washed with detergent for 10 min followed by running tap water for 30 min. Surface sterilization was carried out by treatment with 0.1% mercuric chloride + 0.05% cetrimide + 0.05% bavistin mixture for 5 min and were washed thoroughly with autoclaved distilled water. The seeds were then germinated on Knop's agar media containing 0.8% agar (w/v), devoid of sucrose.

Infection and Disease assessment

Seven days after the germination, 1 ml of freshly prepared inoculum was transferred aseptically to culture-media containing seedlings and incubated under controlled conditions. Ten replicates of each accession were maintained. Evaluation of degree of infection was carried out by measuring the symptoms on roots and shoots on a 0-3 scale for 22 days at a regular interval of 2 days (Lebeda and Buczkowski, 1986; Luhova *et al.,* 2002). The wilt incidence as per centage of diseased plants.

The percentage of mortality was scored as per the method explained by Iqbal *et al.* (2005) using 1-9 scale with rating awarded as follows : 1-highly resistant (0-10% plants wilted); 3-resistant (11-20% plants mortality); 5-moderately resistant (21-30% mortality); 7-susceptible (31-50% mortality) and 9-highly susceptible (more than 50% mortality). Both wilt incidence as well as percentage mortality were considered to categorize the accessions as resistant and susceptible. At the end of 22nd day, the plants were removed from the media and the lengths of root and shoots were measured. The dry weight was measured by incubating the plant parts (root and shoot) overnight in 60^0C in hot air oven. All the readings were subjected to ANOVA and mean separation was achieved based on significant Tukey's HSD test.

DNA Isolation for AFLP analysis: About 1g of fresh leaf material was harvested from 2-3 weeks old seedlings from ten individual plants of each *Mucuna* accessions and bulked. DNA isolation was done using modified Doyle and Doyle (1990) method. The leaf material was ground in liquid nitrogen and then homogenized using extraction buffer containing cetyltrimethylammonium bromide (CTAB), 0.5% charcoal along with 0.2% β-mercaptoethanol and incubated at 60^0C for 1h. Purification steps were carried out twice with chloroform: isoamlyalcohol (24:1) and once with phenol: chloroform: isoamylalcohol (1:1). Finally DNA was pelleted with 0.67 volumes of propanol followed by ethanol (70%) wash. Air dried pellets were re-suspended in 0.5 ml

of 1X Tris- EDTA buffer (pH 8.0) and quantified fluorometrically on 0.8% agarose gel using ethidium bromide staining.

AFLP method: AFLP fingerprinting was carried out as described by Capo-Chichi *et al.* (2001) with some modifications as described below. Genomic DNA (500 ng) was digested with 10 U of *Eco* RI and 4 U of *Mse* I (New England Biolabs, USA) at 37^0C for 3 h. Without inactivating the restriction enzymes, adapters [*Eco* RI (5 pmol) and *Mse* I (50 pmol)] were ligated to the restricted DNA fragments in ligation buffer [1x T4 DNA ligase buffer, 1 µl of T4 DNA ligase (NEW ENGLAND BIOLABS, USA)] and incubated at 37^0C for 12 h. Pre-amplification of the diluted (2-fold), ligated DNA was carried out with primers (complimentary to the *Eco* RI and *Mse* I adapters, with two sets of selective nucleotides, one with cytosine and guanine and the other with adenine and cytosine respectively) in PTC-200^{TM} (MJ RESEARCH INC., USA) thermocycler using the following cycling parameters: 20 cycles of 94°C (denaturation) for 30 s, 56°C (annealing) for 60 s, 72°C (extension) for 60 s. The diluted (4-fold), amplified products were used as the template for selective amplification. The second amplification was carried out with twelve selective primer combinations of *Eco* RI and *Mse* I each with three selective nucleotides in a total volume of 10 µl. The PCR program consisted of two segments. The first segment comprised 12 cycles with one cycle at 94^0C for 30 s, 65^0C for 30 s, and 72^0C for 60 s. The annealing temperature was then lowered by 0.7^0C per cycle during the first 12 cycles to reach an optimum temperature of 56^0C. The second segment comprised 23 cycles at 94^0C for 30 s, 56^0C for 60 s and 72^0C at 60 s.

Gel electrophoresis was carried out using Sequegen DNA sequencer (BIORAD^{TM}, USA). Following the amplification reaction, the PCR products were mixed with 8 µl of formamide stop/loading buffer and denatured at 94^0C for 5 min, then chilled immediately to 4^0C. Eight microliters of each reaction mixture was loaded onto a 6% denaturing PAGE. The gel was pre-run in 1X TBE freshly prepared from a 10X TBE stock solution. The key electrophoresis parameters included voltage set at 1200 V and temperature at 45^0C. The DNA bands were visualized using silver stain (SIGMA ALDRICH INDIA PVT. LTD.).

Polymorphism analysis and construction of phenogram: Each AFLP marker was treated as unit character and scored as a binary code (1/0). The results were analyzed using NTSYS-pc version 2.21(Exerter software; Rohlf 2009) with SIMQUAL option on the basis of Jaccard's coefficient to generate genetic similarity coefficients among all the possible pairs and ordered in similarity matrix (Jaccard, 1908). The resulting matrices were subjected to clustering method by UPGMA (Sokal and Michener 1958). To find the robustness of the phenogram, bootstrapping analysis was carried (1000 replicates) with Winboot software (Yap and Nelson, 1996). The goodness of fit of the clustering to the data matrix was calculated by the COPH and MXCOMP programs.

Results and Discussion

Isolation of Fungus and identification: On Czapek Dox, the isolates grew within 5-7 days forming white aerial mycelia which developed light purple tinge with abundant microconidia on microconidiophores by 10th day (Fig. 1). Microconidia were oval to ellipsoid-cylindrical measuring 5-13 × 2.5-5 µm. Macroconidia were long fusoid to falcate in shape with 3-4 septa, and 17-33×2-5 µm in size. Globose to ellipsoid chlamydospores measuring 3.7-17 × 3.8-17 µm were also sparsely formed. These morphological characteristics of the fungus were in accordance with those of *F. oxysporum* Schlecht. emend. Snyd. and Hans. (Booth 1970; Nelson *et al.* 1983) which was further confirmed by observations on pathogenicity.

Pathogenicity: The isolates produced typical symptoms on susceptible plants. The disease symptoms (Fig. 2) appeared on 8th day after infection with plants developing dark brown lesions on the leaf and at stem-root junction. Yellowing of leaves and defoliation was noticed by 15-18th day and by the end of 22nd day several plants were completely dead. The fungus was re-isolated from these lesions and inoculated to reconfirm the pathogen.

***Screening for resistant accessions*:** Based on wilt incidence 15 accessions were grouped into 5 ranks (Table 2). Six accessions that showed PM of plants more than 50% were grouped as highly susceptible. Four accessions with 31-50% PM were considered as susceptible. Genotype IC385842 with 30% of PM showed moderate resistance to disease. 500108KA was grouped under resistant accessions with PM of 20% and three accessions IC385841, IC471876 and 500101KA were highly resistant to Fusarium wilt with PM of 0-10%. Overall results suggested that about 30% of the genotypes used in the study were resistant to Fusarium wilt disease while nearly 70% showed different degrees of susceptibility.

The dry weights and length of root and stem measured after 22 days showed significant differences among the plants of different disease severity class (Table 3) revealing inherent difference

in response of plants to infection challenges. Tukey's HSD test also supported this (Fig. 3). All the resistant plants registered good survival percentage and healthy growth even after the field transfer following the completion of the experiment.

AFLP analysis and selection of genetically diverse parents: Twelve AFLP primer combinations (Table 4) produced about 1612 fragments of which 1605 were polymorphic with percentage of polymorphism recorded to be about 99%. The genetic distance between all the genotypes based on Jaccard's coefficient ranged from 63 - 87%. Among the accessions that showed contrasting disease phenotypes, 500108KA (Resistant) and IC471870 (susceptible) showed highest genetic distance (87%) followed by 500101KA (Highly resistant) and 500155AP (Highly Susceptible), (79%) based on AFLP data. These two groups are suggested as potential parental lines for mapping Fusarium wilt disease in Velvetbean. Contrasting genotypes other than the above revealed low polymorphism (close similarities) at the genetic level and therefore were not considered suitable as parents for mapping population. The UPGMA cluster analysis (Fig. 4) indicated that the overall grouping of accessions in the present study is mainly based on botanical status of the plants rather than the disease phenotype.

Lack of information on germplasm evaluation in underutilized plant species has been extensively discussed (Phogat *et al.,* 2006; Dawson *et al.,* 2007; Bhattacharjee, 2009). Fusarium wilt, even though is widely reported in major legume species (Varshney et al., 2010) was never accounted in Velvetbean. In this first report, we have confirmed the incidence as well as the causal organism of this disease through detailed study of symptoms, morphological characteristics as well as pathogenicity which were in accordance with those of *F. oxysporum* Schlecht. emend. Snyd. and Hans. (Booth, 1970; Nelson *et al.*, 1983). Nearly 70% of the examined plants showed moderate to high degree of susceptibility to wilt indicating high rate of prevalence of this disease among the cultivated Velvetbean accessions. This calls for an immediate action on initiating disease resistance breeding in this plant.

We have used *in vitro* approach for disease screening. This is preferred over traditional approach, as the latter, based mainly on the field tests, requires a huge experimental area, and often plants are damaged by diseases not-targeted or other climatic factors (Hwang, 1990; Smith *et al.,* 2006). *Fusarium*-tolerant plants have already been obtained using culture filtrates as a selection factor in Alfalfa, *Medicago sativa* and Pineapple, (Arcioni *et al.,* 1987; Binarova *et al.,* 1990; Borrás *et al.,* 2001). The present study further confirms the reliability of this method for screening large number of accessions reducing the time and space required in such efforts.

Categorization of accessions as resistant and susceptible was done in accordance with approaches used in other leguminous species. For instance, genotypes showing <10% (Halila and Strange, 1997) and <20% (Bayaa *et al.,* 1997) wilt incidence of Fusarium wilt were considered resistant even in chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*), respectively.

Several earlier studies in other crops have exploited DNA markers as a tool to screen polymorphism among the parental lines of germplasm collection (Rommens and Kishore, 2000; Xu *et al.,* 2003; Bert *et al.,* 2008). This is the first molecular investigation on the identification of contrasting parents for Fusarium wilt disease in Velvetbean. The genotypes revealed through the present study will form a strong basis for future mapping population aiming at development of Fusarium wilt resistance-specific gene/markers in this plant. Such a tool could enable Velvetbean breeders to tag and follow the inheritance of specific chromosome segments that are linked to resistance traits from natural lines into improved cultivars. The markers could also be useful for defining resistant genotypes while at an early seedling stage without uncertainties due to environmental interactions for disease development and predicting morphological parameters of adult plants.

In conclusion, the identification of Fusarium wilt specific diverse parental lines through *in vitro* screening and AFLP fingerpinting in this study would trigger efforts on disease resistance breeding in Velvetbean. It would also promote use of such simple and cost-effective approach in other under-utilized plant species thus providing a much desired hope and direction for MAS/MAB in these plants.

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References

Aberkane, A.M., Cuenca-Estrella, A., Gomez-Lopez, E., Petrikkou, E., Mellado, A., Monzón, J.L. and Rodriguez-Tudela., the Eurofung Network. 2002.

Comparative evaluation of two different methods of inoculum preparation for antifungal susceptibility testing of filamentous fungi. *J. Antimicrob. Chemother.,* **50:** 719-722.

Altinkut, A., Kazan, K. and Gozukirmizi, N. 2003. AFLP marker linked to water-stress-tolerant bulks in barely (*Hordeum vulgare* L.). *Genet. Mol. Biol.,* **26(1):** 77-82.

Arcioni, S., Pezzotti, M. and Damiani, F. 1987. *In vitro* selection of alfalfa plants resistant to *Fusarium oxysporum* f. sp. *medicaginis*. *Theor. Appl. Genet.,* **74:** 700-705.

Bayaa, B., Erskine, W. and Singh, M. 1997. Screening lentil for resistance to Fusarium wilt: methodology and sources of resistance. *Euphytica.,* **98:** 69–74.

Bell, E.A. and Janzen, D.H. 1971. Medical and ecological considerations of L-Dopa and 5- HTP in seeds. *Nature,* **229:**136-137.

Berner, D.K., Killani, A.S., Aigbokban, E. and Couper, D.C. 1992. *Macrophomina phaseolina* on tropical cover crop *Mucuna pruriens* var. *utilis*. *Plant. Dis.,* **76:**1283.

Bert, C.Y., Collard, Casiana, M., Vera Cruz, Kenneth, L. McNally, Parminder, S. Virk, David and J. Mackill. 2008. Rice molecular breeding laboratories in the Genomics era: current status and future considerations. *Int. J. Plant. Genomics,* pp 25.

Bhattacharjee, R.. 2009. Harnessing Biotechnology for conservation and increased utilization of orphan crops. *ATDF. J.*, **6(3/4):** 24-83.

Binarova, P., Nedelnik, J., Fellner, M. and Nedbalkova, B. 1990. Selection for resistance to filtrates of *Fusarium* spp. in embryogenic cell suspension culture of *Medicago sativa* L. *Plant. Cell. Tiss. Org.,* **22:**191-196.

Booth, C. 1970. *Fusarium oxysporum*. CMI Descriptions of Pathogenic Fungi and Bacteria. Kew, Surey, England, No. 211.

Borrás, O., Santos, R., Matos, A.P., Cabral, R.S. and Arzola, M. 2001. A first attempt to use a *Fusarium subglutinans* culture filtrate for the selection of pineapple cultivars resistant to fusariose disease. *Pl. Breeding,* **120:** 435-438.

Buckles, D. 1995. Velvetbean: a 'new' plant with a history. *Econ. Bot.,* **49:**13-25.

Capo-chichi, L.J.A., Weaver, D.B. and Morton, C.M. 2001. AFLP assessment of genetic variability among Velvetbean (*Mucuna* sp.) accessions. *Theor. Appl. Genet.,* **103:**1180-1188.

Cvikrová, M., Binarová, P., Eder, J. and Nedělník, J. 1992. Accumulation of phenolic acids in filtrate-treated alfalfa cell cultures derived from genotypes with different susceptibility to *Fusarium oxysporum*. *J. Plant. Physiol.,* **140:** 21-27.

Dawson, I.K., Guarino, L. and Jaenicke, H. 2007. Underutilized plant species: Impacts of promotion on biodiversity. International Centre for Underutilized Crops Position Paper No. 2, Internationl Centre for Underutilised Crops, Colombo, Sri Lanka.

Doyle, J.J. and Doyle, L.J. 1990. Isolation of DNA from plant tissue. *Focus,* **12:**13-15.

Evans, D.A. and Sharp, W.R. 1986. Applications of somaclonal variation. *Biotech.,* **4:** 528-532.

Giovanni, C.D., Orco, P.D., Bruno, A., Ciccarese, F., Lotti, C. and Ricciardi, L. 2004. Identification of PCR-based markers (RAPD, AFLP) linked to a novel powdery mildew resistance gene (ol-2) in tomato. *Pl. Sci*., **166:** 41-48.

Halila, M.H. and Strange, R.N. 1997. Screening of Kabuli chickpea germplasm for resistance to fusarium wilt. *Euphytica.* **96:**273–279.

Haq, N. 1983. New Food legume crops for the tropics. In: Nugent J. and Cormor (ed.) Better crops for the food, Cuba Foundation Symposium, M O Pitman Books, London, pp 144-160.

Huang, Y.H. and Hartman, G.L. 1998. Reaction of selected soybean genotypes to isolates of *Fusarium solani* f. sp. *glycines* and their culture filtrates. *Plant. Dis.,* **82:** 999-1002.

Hwang, S.C. 1990. Somaclonal resistance in Cavendish banana to Fusarium wilt. In: Ploetz RC (ed) Fusarium Wilt of Banana. APS Press, St. Paul, Minnesota, USA, pp 121-125.

Infantino, A. Porta-Puglia, A. and Singh, K.D. 1996. Screening wild *Cicer* species for resistance to Fusarium wilt. *Plant. Dis.,* **80:** 42-44.

Iqbal, M.S., Haq, A., Baksh, A. Ghafoor and Haqqani, A.M. 2005. Screening of chickpea genotypes for resistance against Fusarium wilt. *Mycopath.,* **3**(1and2):1-5.

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. *Bull. Soc. Vaud. Sci. Nat.,* **44:** 223–270.

Jain, A., Ariyadasa, R., Kumar, A., Srivastava, M.N., Mohan, M. and Nair, S. 2004. Tagging and mapping of a rice gall midge resistance gene, *Gm8*, and development of SCARs for use in marker-aided selection and gene pyramiding. *Theor. Appl. Genet.,* **109:** 1377– 1384.

James, C.M., Clarke, J.B. and Evans, K.M. 2004. Identification of molecular markers linked to the mildew resistance gene *Pl-d* in apple. *Theor. Appl. Genet.,* **110:** 175–181.

Karp, A. and Edwards, K.J. 1997. Molecular techniques in the analysis of the extent and distribution of genetic diversity. In: Ayad WG, Hodgkin T, Jaradat A, Rao VR (eds) Molecular genetic techniques for plant genetic resources, Proceeding of IPGRI workshop, 9-11, October 1995, International plant genetic resources institute, Rome, pp 11–38.

Keinath, A.P., Howard, F. Harrison, Paul, C. Marino, D. Michael Jackson, Thomas and C. Pullaro. 2003. Increase in populations of *Rhizoctonia solani* and wirestem of collard with velvet bean cover crop mulch. *Plant. Dis.,* **87**: 719-725.

Kim, G.H., Jae-Seoun Hur, Woobong Choi and Young Jin Koh. 2005. Fusarium Wilt of Winter Daphne (Daphne odora Thunb.) caused by *Fusarium Oxysporum. Plant. Pathol. J.,* **21(2):**102-105.

Kraft, J.M. 1994. Fusarium wilt of peas (a review). A*gronomie.,* **14:**561-567.

Kumar, L.S. 1999. DNA markers in plant improvement: an overview. *Biotechnol. Adv.,* **17:** 143-182.

Lebeda, A. and Buczkowski, J. 1986. *Fusarium oxysporum, Fusarium solani* – Tube test. In: Lebeda A (ed) Methods of Testing Vegetable Crops for Resistance to Plant Pathogens. VHJ Sempra, VŠÚZ Olomouc, Czech Republic, pp 247-249.

Lebeda, A., and Švábová, L. 1997. Variation in response of several wild *Pisum* spp. to *Fusarium solani* and *Fusarium oxysporum*. *Cereal. Res. Commun.,* **25:** 845-846.

Luhová, L., Hedererová, D., Lebeda, A. and Peč, P. 2002. The influence of *Fusarium solani* on enzyme activity of *Pisum sativum* cultivars. *J. Plant. Dis. Protect.,* **109:**113-128.

Najimi, B.N., Boukhatem, S.E., Jaafari, M. Jlibène., Paul, R., and Jacquemin, J.M. 2002. Amplified fragment length polymorphism analysis of markers associated with H5 and H22 Hessian fly resistance genes in bread wheat. *Biotechnol. Agron. Soc. Environ.,* **6:** 79–85.

Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. 1983. *Fusarium* Species, an Illustrated Manual for Identification.The Pennsylvania State University Press, University Park, USA

Phogat, B.S., Dua, R.P. and Arora, R.K. 2006. Germplasm Introduction of Underutilized and New Crops. *Ind. J. Plant*. *Enet. Res.,* **19(3).**

Rich, J., Wright, D., Marois, J. and Sprenkel, D. 2003. Selected Legumes Used As Summer Cover Crops. http://edis.ifas.ufl.edu/IN483, pp 6.

Rohlf, F.J. 2009. NTSYS-pc Numerical Taxonomy and Multivariate Analysis System. Version 2.21, Setauket, New York.

Rommens, C.M. and Kishore, G.M. 2000. Exploiting the full potential of disease resistance genes for agricultural use. *Curr. Opin. Biotechnol.,* **11:**120-125.

Smith, M.K., Hamill, S.D., Langdon, P.W., Giles, J.E., Doogan, V.J. and Pegg, K.G. 2006. Towards the development of a Cavendish banana resistant to race 4 of Fusarium wilt: gamma irradiation ofmicropropagated Dwarf Parfitt (*Musa* spp., AAA group, Cavendish subgroup). *Aust. J. Exp. Agric.,* **46:**107-113.

Sokal, R. and Michener, C. 1958. A statistical method for evaluating statistical relationships. *Kans. Univ. Sci. Bul.,l* **38:**1409–1438.

Sridhar, V. and Rani, B.J. 2004. Record of arthropod pests on velvet bean, *Mucuna pruriens* var. *utilis* under Bangalore conditions. *J. Med. Arom. Plant. Sci.,* **26(3):** 505-506.

Švábová, L. and Lebeda, A. 2005. *In vitro* selection for improved plant resistance to toxin-producing pathogens. *J. Phytopathol.,* **153:**52-64.

Trigiano, R.N., Windham, M.T. and Windham, A.S. 2004. Concepts and Laboratory Exercises. *Plant. Pathol.* CRC Press, Boca Raton, Florida, USA, pp 413.

Varshney, R.K., Mahendar Thundi, Greg, D. May and Scott, A. Jackson. 2010. Plant Breeding Reviews: Legume Genomics and Breeding. John Wiley and Sons Ltd, pp 257.

Venkatachalam, L., Sreedhar, R.V. and Bhagyalakshmi, N. 2008. The use of genetic markers for detecting DNA polymorphism, genotype identification and phylogenetic relationships among banana cultivars. *Mol. Phyl. Evo.,* **47:** 974-985.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic. Acids. Res.,* **23:**4407–4414.

Xu, Y., Kang, G. and Zhang, H. 2003. Genetic Assessment of Watermelon Germplasm using the AFLP Technique. *Hort. Sci.,* **38(1):**81-84.

Yap, V.I. and Nelson, R.J. 1996. WINBOOT- A Program for Performing Bootstrap Analysis of Binary Data to Determine the Confidence Limits of UPGMA-Based Dendrograms.- IRRI Discussion paper series No. 14, Manila, Philippines; International Rice Research Institute

Table 1. List of *Mucuna pruriens* var. *utilis* genotypes used in the study

Sl.No	Accession No.	Place Of Collection
1	IC369144	NBPGR (Latehar, Jharkhand)
2	IC392241	NBPGR (Dhanbad, Jharkhand)
3	IC385926	NBPGR (Dhangadih, Jharkhand)
4	IC185926	NBPGR (New Delhi)
5	500155AP	Tandur, Andhra Pradesh
6	IC326953	NBPGR (Jarengi, Solan, Himachal Pradesh)
7	500102KA	Bangalore, Karnataka
8	IC471870	NBPGR (New Delhi)
9	500159PY	Madagadipet, Tamil Nadu
10	IC385928	NBPGR (New Delhi)
11	IC385842	NBPGR (Mohanpur, Jharkhand)
12	500108KA	Hunasamaranhalli , Bangalore, Karnataka
13	500101KA	Bangalore, Karnataka
14	IC471876	NBPGR (New Delhi)
15	IC385841	NBPGR (Pakud, Jharkhand)

Table 2. Distribution of genotypes in various disease severity groups based on wilt incidence

Sl.No	Accession No.	% of Wilt incidence	Type*
1	IC369144	80	HS
2	IC392241	80	HS
3	IC385926	80	HS
4	IC185926	70	HS
5	500155AP	70	HS
6	IC326953	60	HS
7	500102KA	50	S
8	IC471870	50	S
9	500159PY	40	S
10	IC385928	40	S
11	IC385842	30	MR
12	500108KA	20	R
13	500101KA	10	HR
14	IC471876	10	HR
15	IC385841	0	HR

* HS- Highly susceptible; S- Susceptible; MR- Moderately resistant;
R- Resistant; HR- Highly resistant

Table 3. Variations in Root –Shoot parameters of different accessions for *Fusarium oxysporum* challenging

Sl.No.	Accession No	Root length (cm) Mean ± SD [y]	Dry root weight(g) Mean ± SD [y]	Stem length (cm) Mean ± SD [y]	Dry stem weight (g) Mean ± SD [y]	Type*
1	500108KA	24.95±1.174[A]	0.904±0.079[A]	32.36± 2.26[A]	3.42±0.85[B]	R
2	IC385841	22.73±0.790[AB]	0.793±0.065[AB]	29.58±1.23[AB]	3.57±1.96[B]	HR
3	500101KA	22.26±0.908[B]	0.760±0.053[B]	29.32±0.96[B]	4.26±0.55[A]	HR
4	IC471876	20.60±0.536[B]	0.619±0.027[C]	28.54±1.22[B]	3.98±0.27[A]	HR
5	500159PY	14.85±1.484[C]	0.546±0.075[CD]	18.66±0.98[C]	2.93±0.64[C]	S
6	IC385926	8.70±2.980[D]	0.260±0.094[E]	12.23±0.14[D]	1.57±1.02[D]	HS
7	500102KA	14.06±1.620[C]	0.447±0.053[D]	18.27±1.03[C]	2.45±1.62[C]	S
8	IC385842	19.76±1.274[B]	0.600±0.155[C]	24.66±0.58[B]	3.42±1.22[B]	MR
9	IC471870	13.63±1.767[D]	0.283±0.049[E]	19.14±0.87[C]	2.86±0.73[C]	S
10	IC185926	9.19±1.502[D]	0.262±0.089[E]	10.32±0.54[D]	1.27±0.36[D]	HS
11	IC385928	14.32±1.948[C]	0.484±0.072[D]	16.88±0.65[D]	2.78±0.36[C]	S
12	500155AP	7.90±1.175[D]	0.238±0.062[E]	11.77±1.48[D]	1.49±0.93[D]	HS
13	IC326953	7.68±1.450[D]	0.231±0.043[E]	11.93±0.26[D]	1.68±1.01[D]	HS
14	IC369144	7.21±1.786[D]	0.206±0.085[E]	10.69±1.20[D]	1.99±0.28[D]	HS
15	IC392241	7.00±1.320[D]	0.168±0.031[E]	12.12±0.99[D]	1.46±0.79[D]	HS

[Y]Values are mean ± standard deviation of 10 independent experiments. Means followed by same letter are not significantly different at 1% significance level as determined by Tukey's HSD test

Table 4. Sequences of Oligonucleotide adapters and primers used in AFLP

Name	Code	Sequence
***Eco* RI adapter**	E-0	5'-AAT TGG TAC GCA GTC TAC-3'
		3'-CC ATG CGT CAG ATG CTC-5'
***Mse* I adapter**	M-0	5'-TAC TCA GGA CTC AT-3'
		3'-G AGT CCT GAG TAG CAG-5'
***Eco* RI primer**	E-A00	5'-GAC TGC GTA CCA ATT C A-3'
***Mse* I primer**	M-C00	5'-GAT GAG TCC TGA GTA A C-3'
***Eco* RI primer**	E-C00	5'-GAC TGC GTA CCA ATT C C-3'
***Mse* I primer**	M-G00	5'-GAT GAG TCC TGA GTA A G-3'
***Eco* RI + 3-CAC**	E-CAC	5'- GAC TGC GTA CCA ATT C CAC-3'
***Eco* RI + 3-CAA**	E-CAA	5'-GAC TGC GTA CCA ATT C CAA-3'
***Eco* RI + 3- ACT**	E-ACT	5'-GAC TGC GTA CCA ATT C ACT-3'
***Eco* RI + 3- AAC**	E-ACC	5'-GAC TGC GTA CCA ATT C AAC-3'
***Mse* I + 3-GCT**	M-GCT	5'-GAT GAG TCC TGA GTA A GCT-3'
***Mse* I + 3-GCA**	M-GCA	5'-GAT GAG TCC TGA GTA A GCA-3'
***Mse* I + 3-CAT**	M-CAT	5'-GAT GAG TCC TGA GTA A CAT-3'
***Mse* I + 3-CAG**	M-CAG	5'-GAT GAG TCC TGA GTA A CAG-3'
***Mse* I + 3-CTA**	M-CTA	5'-GAT GAG TCC TGA GTA A CTA-3'
***Mse* I + 3-CTC**	M-CTC	5'-GAT GAG TCC TGA GTA A CTC-3'

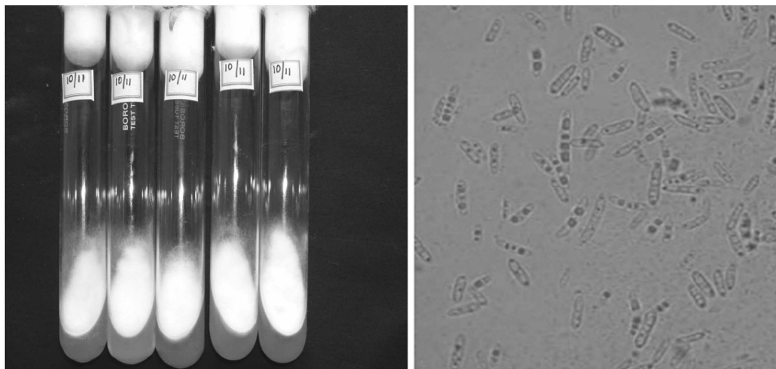
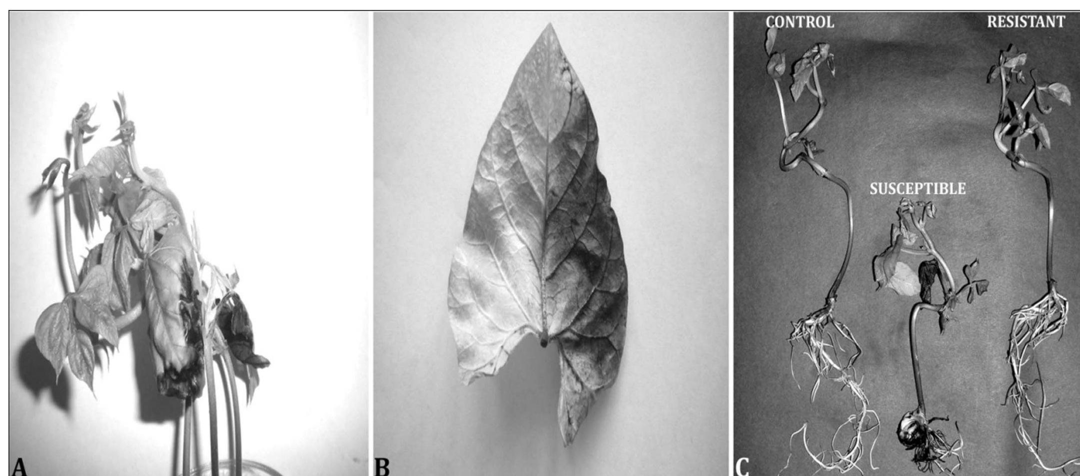


Fig. 1 Macroscopic and microscopic view of *Fusarium oxysporum*



A- Wilting; B- Chlorosis of leaves; C- Growth depression of both root and shoot

Fig. 2 Symptoms of Fusarium wilt on susceptible plant observed after 22 days

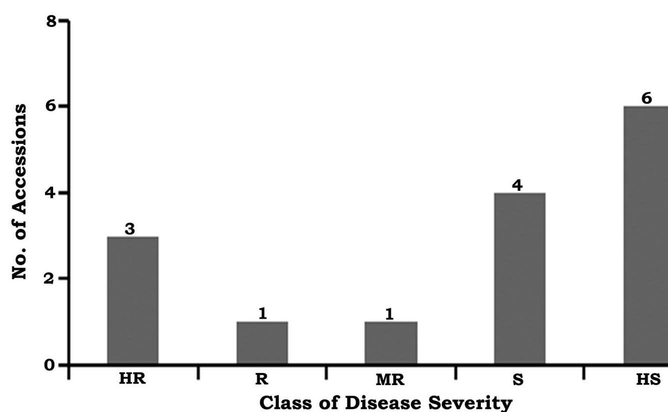


Fig. 3 Distribution of accessions in different disease severity class based on Tukey's HSD test

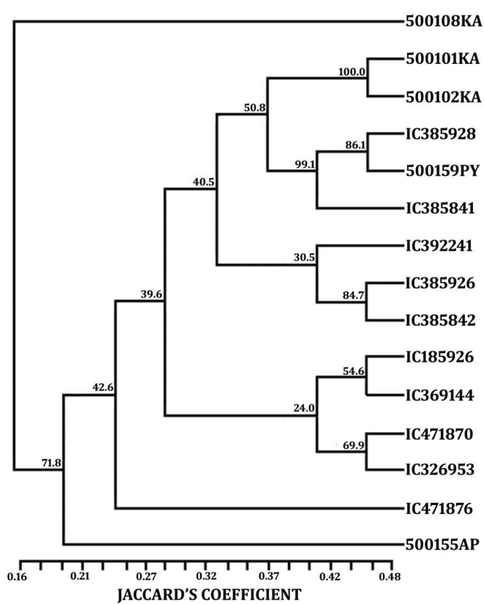


Fig. 4 UPGMA phenogram of 15 *Mucuna* accessions based on AFLP fragments analysis. The values on the nodes of the cluster indicate the bootstrap values and the scale represents Jaccard's similarity coefficient values