Genetic Inheritance of Fusarium Wilt Resistance in Pigeon Pea (*Cajanus cajan* (L.) Millsp.) Genotypes

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Abstract: *Fusarium wilt disease (FWD) caused by F. udum is the most serious disease of pigeon pea [Cajanus cajan (L.) Millsp.]. Significant yield losses which could result up to 100% were reported in susceptible cultivars throughout the pigeon peas growing areas. The reported loss results from low information on gene responsible with fusarium wilt resistance and its mode of inheritance. A study on genetic inheritance of Fusarium wilt resistance, was conducted on susceptible and resistant parents [F_1, F_2, (BC_1F_1), (BC_2F_1), and ICEAP 00048 and ICPL 96061]. The observation on F_2 generation showed a ratio of 3:1 that agreed with Mendelian ratio. It was concluded that the resistance to Fusarium wilt is under the influence of dominant allele hence it could be used to donate gene for disease resistance into genotypes where Fusarium wilt is of an economic problem.*

Key words: Pigeon peas, Fusarium wilt

Introduction

Pigeonpeas *Cajanus cajan* (L.) Millspaugh are normally grown as an annual shrub or more usually short-term perennial shrub that may reach 4-5m in height, but usually 1-2m only, woody at the base, with a variable habit, but usually erect, with deep and quick growing tap root. Angular stem resulting from three ribs starting from the base of each petiole. The crop has trifoliate leaves, alternate set in a spiral along the stem. Leaflets are oblong, lanceolate, 5-10 cm long x 2-4 cm wide, pubescent likewise the stem. Lateral petioles, 2-3 mm the terminal one reaching 10-20 mm. Stipules are linear 2-3 mm long, stipulets filiform 1-2 mm long. Flowers usually yellow but they may also be striated with purple streaks or plain red, corolla 20-25 mm, with the flag 18-20 mm wide. Calyx 10-12 mm long, with 5 linear teeth. Inflorescence composed of racemes having 5-10 flowers on top of an axillary, little divided peduncle. Pods flat, with an acuminate tip, pubescent and of variable colour, 5-9 cm long x 12-13 mm wide, containing 2-9 seeds in shades of brown, red or black (Osman *et al*., 2012).

Importance and Uses of Pigeonpeas

Pigeonpea is an important food in developing tropical and subtropical countries (Vange and Moses, 2009). Its major benefits include food for human consumption (proteins, carbohydrates, minerals) and income generation (Shiferaw *et al*., 2007; Kunjeku and Gwata, 2011; Infonet-biovision, 2013). The protein content of commonly grown pigeon pea cultivars ranges between 17.9 and 24.3 g/100g for whole grain samples, and between 21.1 and 28.1 g/100 g for split seed (Sheahan, 2012). Wild species of pigeon pea have been found to be a very promising source of high-protein and several genotypes were developed with a protein content as high as 32.5% (Singh *et al*., 1990).

The high-protein genotypes also contain significantly higher (about 25%) sulphur-containing amino acids, namely methionine and lysine which assist in break down of fats and thereby prevents the build-up of fat in the arteries, as well as assisting with the digestive system (Oluwaseun, 2013). Pigeon pea seeds contain about 57.3 to 58.7% carbohydrate, 1.2 to 8.1% crude fibre, and 0.6 to 3.8% lipids (Hassan *et al*., 2013). It is also a good source of soluble vitamins thiamine, riboflavin, niacin, and choline (Valenzuela, 2007). Since pigeon peas contain high protein value they supplement the diets for millions of people, especially traditional cereal-banana- or tuber-based diets of resource-poor farmers that are generally protein-deficient. The perennial nature of pigeon pea allows farmers to take multiple harvests with surpluses traded in both local and international markets (Odeny, 2006).

Pigeonpea is an important component in the integrated crop and livestock systems of the semi-arid tropics as it's by-products of split and shrivelled seed are used as livestock feed (Troedson *et al.,* 1990). The present high cost of animal sources of protein feeds, such as fish and bone meal, makes pigeonpea ideal to be used as a good plant protein substitute as it is less expensive. Due to its role as excellent forage/ feed for livestock there is a great scope for selecting cultivars with not only higher grain yields but also higher forage yields and crude protein (Morton, 1976). Pigeonpea produces more nitrogen from plant biomass per unit area of land than many other legumes although it usually produces fewer nodules than legumes. The crop can fix about 70 kg N/ha per season by symbiosis until the mid-pod-fill stage. This is around 88% of the total nitrogen content of the plant at that stage of growth. The residual effect on a following cereal crop can be as much as 40 kg N/ha (Phatak *et al.,* 1993).

Fusarium wilt Disease in Pigeonpeas

Pigeonpea is attacked by more than 100 pathogens including fungi, bacteria, and viruses, nematodes and mycoplasma-like organisms, but *Fusarium udum* is considered the most important soil borne pathogen of pigeonpea (Parde *et al*., 2012; Sharma, 2013; Chhetry and Devi, 2014). Fusarium Wilt Disease caused by fungal pathogen *Fusarium udum* Butler, is the vascular disease that travel through seed and soil (Pande *et al*., 2007) which is difficult to handle through chemical, biological and other cultural practice like crop rotation, and field sanitation.

It is the most devastating seed and soil borne disease of pigeon pea affecting plants at all stage of growth eventually causing significant yield losses in susceptible cultivars throughout the pigeon pea growing areas (Kiprop *et al*., 2005; Karimi *et al*., 2010; Datta and Lal, 2012). In India, the annual loss due to this disease is estimated at US $71 million (Reddy *et al*., 1993). Fusarium wilt causes economic loss in pigeonpea of about 470, 000 t of grains in India and 30,000 t of grains in Africa. Karimi *et al*. (2012) reported wilt incidence (and range) in Kenya, Malawi and Tanzania of 15.9% (0-90%), 36.6 (0-90) and 20.4% (0-60%) respectively with annual loss estimated at US $ 5 million in each of the countries. According to Mbwaga (1995) in Tanzania, an incidence of *Fusarium* wilts as high as 96% has been observed.

The disease can occur at any stage of the crop with the entry of fungus into the hosts' vascular system at root tip leading to progressive chlorosis of leaves, branches and finally wilting of the whole plant. After infestation in the soil of some areas it can rapidly spread to new areas (Prasanthi *et al*., 2009). The information on gene responsible for Fusarium wilt resistance of pigeon peas is still inadequate. In order to breed for fusarium wilt resistance on pigeon pea it is important to understand gene responsible for resistance and its mode of inheritance. Therefore, the objective of this experiment was to determine the gene responsible for Fusarium wilt and its mode of genetic inheritance in pigeon peas in order to control significant yield losses caused by *F.udum* (Plate 1).

Plate 1: Partial wilting in pigeon pea caused by infection of* Fusarium udum *pathogen

Methodology

Several populations including susceptible and resistant parents [F_1, F_2, (BC_1F_1), (BC_2F_1), and ICEAP 00048 and ICPL 96061] were planted in the screen house at Sokoine University of Agriculture. After seven days from germination, the individual F_1, F_2, BC_1F_1, BC_2F_1 and parents were uprooted from the sand and shaken to remove the excess sand and washed in sterile dH_2O. The seedlings were immersed in sterile distilled water to remove sand from the roots and by using sterile scissors; one cm of the distal end of the root system was cut.

Using root tipping inoculation techniques the seedlings were dipped in the inocula at a concentration of 1.0×10^6 conidia ml^{-1} and suspended for 10 minutes to allow the conidia to enter the wounds created on the root systems. The plants were then transplanted into pots containing mixture of sterile soil and sand (3:1 v/v) while arranged in Randomized Complete Design. Watering was done after transplanting in an interval of two days.

Data Collection and Analysis

The number of wilted and health plants were recorded and chi-square test was performed to test the goodness of fit between the theoretical expected and observed ratios of resistant and susceptible plants. It was

hypothesized that the R:S ratio fits 3:1. The Chi-square test was computed using the following formula:

$$\chi^2 = \sum \frac{[Observed - Expected]^2}{Expected}$$

Where, χ^2 = calculated chi-square value
Observed = frequency observed
Expected = frequency expected

Results

The resistant parents (ICPL 96061) used in this study showed no reaction to EMKM2 while susceptible parents (ICEAP 00048) showed high level of virulence as 60 plants were all susceptible to the same inoculum (rr) used in resistant genotype. The F_1 progenies developed from the crosses of resistant × susceptible and their reciprocals were all resistant to FWD.

The number of resistant and susceptible progenies obtained in F_2 populations derived from crosses between resistant parent ICPL 96061 and susceptible ICEAP 00048 ($\chi^2_{(0.05)}$ = 0.72, P ≥ 0.639) demonstrated a ratio of 3:1 (resistant : susceptible). All the BC_1F_1 (backcrosses to the resistant parents) showed a resistant reaction while their respective BC_2F_1 segregated into a ratio of 1:1 (Table 1, Figure 1).

Table 1: *Genetic analysis of resistance to FWD in pigeon pea* (**Cajanus cajan**) *populations derived from ICEAP 00048 × ICPL 96061*

Pedigree	Generations	Observed frequencies: Total plants	R	S	Ratio R: S	χ^2	χ^2 Tabulated
Crosses using ICEAP 00048 and ICPL 96061 as parents							
ICEAP 00048	P1	60		60			
ICPL 96061	P2	60	60				
ICEAP 00048× ICPL 96061	F_1	80	80				
ICPL 96061× ICEAP 00048	F_1	80	80				
ICEAP 00048× ICPL 96061	F_2	206	159	47	3. 38:1	0.72	3.84
ICPL 96061× ICEAP 00048	F_2	206	157	49	3.20:1	0.35	3.84
ICEAP 00048× ICPL 96061*[2]	BC_1F_1	60	60				
ICPL9606×ICEAP00048 *[2]	BC_1F_1	54	49	5	9.8:1		
ICEAP00048×ICPL 96061*[1]	BC_2F_1	53	33	20	1.65:1	3.56	3.84
ICPL 96061× ICEAP 00048*[1]	BC_2F_1	54	33	21	1.57:1	3.20	3.84

(A)

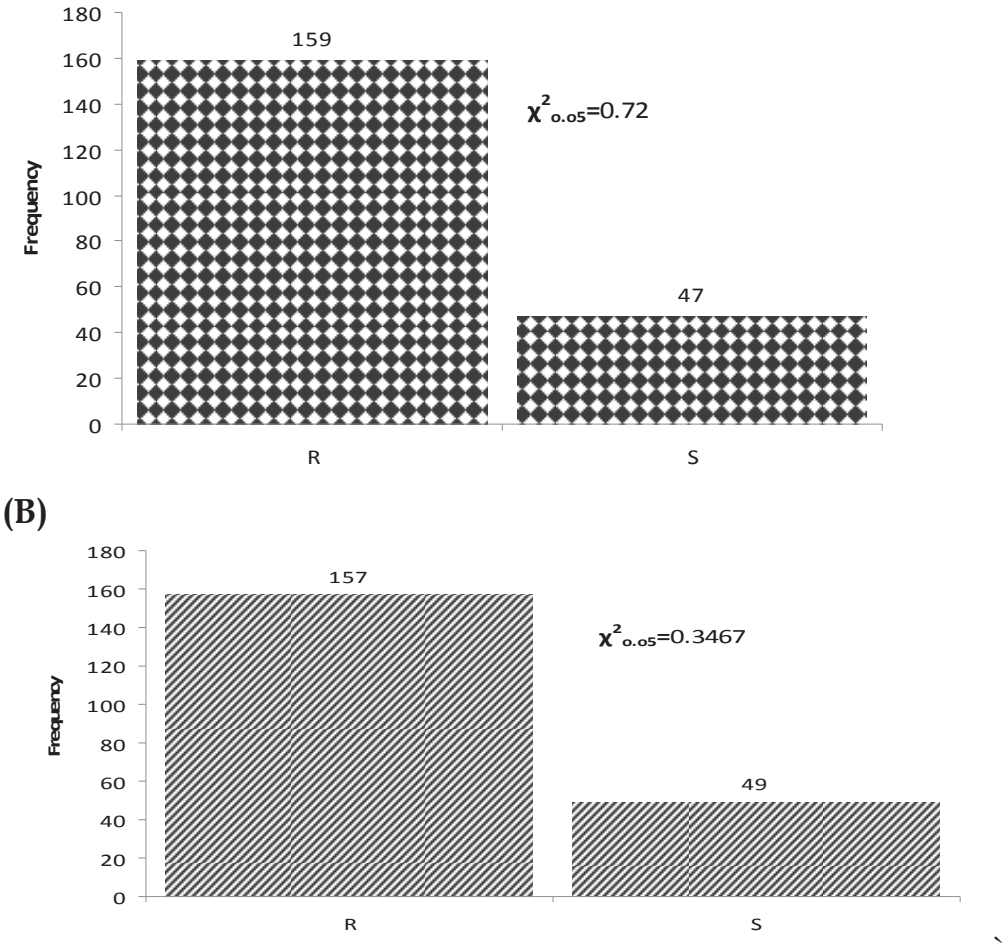

Figure 1: Segregation of F2 pigeon pea (*Cajanuc cajan*) populations derived from (A) ICEAP 0048 × ICPL 96061 (B) ICPL 96061 × ICEAP00048 against EMKM2 isolate

Discussion

The results obtained showed no reaction to EMKM2 isolates in all resistant progenies while all susceptible genotypes were highly virulent to EMKM2 isolate. In F_2 generation pathogenicity test, the observation showed a 3:1 ratio that agreed with Mendelian ratio, thus the resistance to Fusarium wilt is under control of single dominant gene. This observation is in agreement with reports of Karimi *et al*. (2010) that resistance to Fusarium wilt is controlled by single dominant gene and recessive gene. Also, in field beans for example, resistance to Fusariumwilt (*Fusarium oxysporium* Schlechtend f.sp. *phaseoli*) was reported to be controlled by major genes among germplasm of races Durango (Karimi *et al*., 2012). However, Odeny *et al*. (2001); Kumar *et al*. (2009) reported different observation that resistance to Fusariumwilt (Kiboko isolate) in pigeon pea is controlled by recessive genes; a single

recessive gene in cultivar ICEAP00040 of East African origin and duplicate recessive genes in the Indian resistant source, ICP8863, while polygenes controlled resistance in the Mesoamerican types (Odeny *et al.*, 2001).

Conclusion and Recommendation

The study of genetic inheritance in pigeon pea revealed ratio of 3: 1 hence concluded single dominant control. This single dominant could be used to donate gene for disease resistance into genotypes where Fusarium wilt is of an economic problem.

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