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Research Article

Morphological characterization and pollen –pistil interaction in selected genotypes of sunnhemp (*Crotalaria juncea* L.)

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Abstract
Thirty six morphological characters consisting of sixteen biometrical and twenty qualitative characters were studied in six genotypes of sunnhemp (*Crotalaria juncea* L.), a fibre cum green manure crop of the tropics. Of the six genotypes, CO 1 was the earliest to flower while, JRT 610 was the last one to flower. Petal patternings were observed only on the standard petals of SUIN 037 and CO 1. SUIN 053 out-yielded all other genotypes for seed yield. The stigma receptivity was found to vary between genotypes at different stages of flower development. It was very low in K 12 Yellow up to the stage of flower showing initiation of the emergence of standard petal and in other genotypes, stigma receptivity was either low or medium in the same stage. However, it was high or very high in fully opened flowers of all the genotypes. When the pollen grains of five genotypes namely SUIN 053, SUIN 037, K 12 Yellow, JRT 610, CO 1 were used for pollinating the genotype SN 23, the pollen tube growth of SUIN 037 was the fastest to reach the ovary of SN 23.

Key words:*Crotalaria juncea,* Sunnhemp, Floral characters, Pollen germination, Pollen-pistil interaction

INTRODUCTION

Sunnhemp (*Crotalaria juncea* L.), 2n=16, a member of the family Fabaceae is commonly known as Indian hemp, Madras hemp or Brown hemp (Sarkar *et al.,* 2015). The fibre obtained from the stem of sunnhemp plant is slightly lignified, light in colour, somewhat coarse, strong and lasting. It is cultivated as a multipurpose legume and grown especially for its fine fibre and also as green manure (Gupta and Prakash, 1969). The genus *Crotalaria* derived its name from the phenomenal noise made by shaking seeds in a mature pod. In Sanskrit literature of India as early as 400 B.C.E, there is a reference of sunnhemp with the name *Sana.* The fibre is used for various purposes like making paper, fishing nets, mats, strings, twines, flooring mats etc (Chaudhary *et al*., 2015). The allelopathic effects of *Crotalaria juncea* had been documented on weeds, vegetable crops and cover crops in greenhouses and growth chambers (Skinner *et al.,* 2012). Apart from this, the plants were also grown in poor and newly reclaimed soil and were also used for nematode deterrence (Rotar and Joy, 1983). It's been found out that incorporating *C. juncea* along with animal manure, was found to be economical and also productive for the plant (Oliveira *et al.,* 2007). Due to the availability of cheap, synthetic fibres, and the requirement of water bodies for retting and labour intensive nature of its processing, its demand is fast declining (Sarkar *et al*., 2015).

MATERIALS AND METHODS

The study was carried out at the Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University,

Coimbatore during the year 2020-2021, located at an elevation of 27 MSL and 11°N latitude and 77°E longitude. Six genotypes namely, SUIN 053, SUIN 037, K 12 Yellow, JRT 610 were obtained from ICAR-CRIJAF Barrackpore, West Bengal, and SN 23 and CO 1 were obtained from Tamil Nadu Agricultural University were raised in four replications. The crop was grown in a 2 x 2 m plot with 45 cm space between rows, while the genotype SN 23 was grown in an insect cage to prevent any chances of outcrossing.

The flowers of *Crotalaria juncea* were typically Fabaceae type, they were small and colourful normally with golden yellow petals. The most successful pollinator of sunnhemp flower was *Xylocopa pubescens* (Rakesh *et al.*, 2019). Stamens were 10 in number and show dimorphism with five heart shaped and five globose shaped anthers. The biometric characters were subjected to statistical analysis as per Panse and Sukhatme (1954).

The growth characters of the plant along with pod and seed characters were recorded at maturity, while the floral characters were studied by observing ten flowers each from different flowering stages namely 1) Fully mature unopened buds 2) Initiation of the emergence of standard petal 3) Fully emerged standard petal 4) Completely opened flowers **(Fig.1)** of the six genotypes. To determine stigma receptivity, five flowers at each flowering stage were randomly collected between 8 am and 6 pm. Stigma receptivity was measured by dipping the stigmas in 6 per cent hydrogen peroxide solution, stigmas that generated bubbles under 3 minutes were deemed receptive (Zeisler, 1938) **(Fig.2).** Pollen viability at various stages of flowering was tested by using the acetocarmine staining method (Dafni *et al.*, 2005), and viewed under a microscope. Those pollen grains which absorbed and retained the stain were considered to be viable, while the unstained were considered as nonviable. *In vitro* pollen germination and viability, studies were carried out by keeping the pollen grains in 15 per cent sucrose solution containing 200 mg of boric acid, 100 mg of calcium nitrate, 100 mg of magnesium sulphate and 100 mg of potassium nitrate (Brewbaker and Kwack, 1963).

The *in vivo* pollen tube growth studies were carried out by crossing of SN 23 plants in cages with the pollen of five other genotypes and fixing the pollinated pistil of SN 23 in fixative (3:1 concentration of 70% ethanol and glacial acetic acid) followed by keeping in 8 M sodium hydroxide for 6-8 hours under laboratory conditions and staining with 0.1% aniline blue. The samples were viewed under a fluorescent microscope (Nikon Eclipse N*i*-U, made in Japan) using filters 330 nm-380 nm excitation filter. Images were captured by using a Nikon DS-Fi3 camera involving NIS-Elements F Ver 4.60.00 image processing platform. The length of pollen tubes of five genotypes on crossing with SN 23 after different hours of pollination was recorded using the software Image view. The germination of pollen grains of all the six genotypes under *in vitro* conditions in an artificial medium at different hours was also observed and recorded.

RESULTS AND DISCUSSION

In the present investigation, a total of 36 morphological characters consisting of sixteen biometrical (**Table 1**) and twenty qualitative characters (**Table 2**) were recorded in six sunnhemp genotypes namely SN 23, SUIN 053, SUIN 037, K 12 Yellow, JRT 610 and CO 1. The plant height at maturity of the six genotypes varied from 143.6 to 184.4 cm with SUIN 053 being comparatively dwarf at

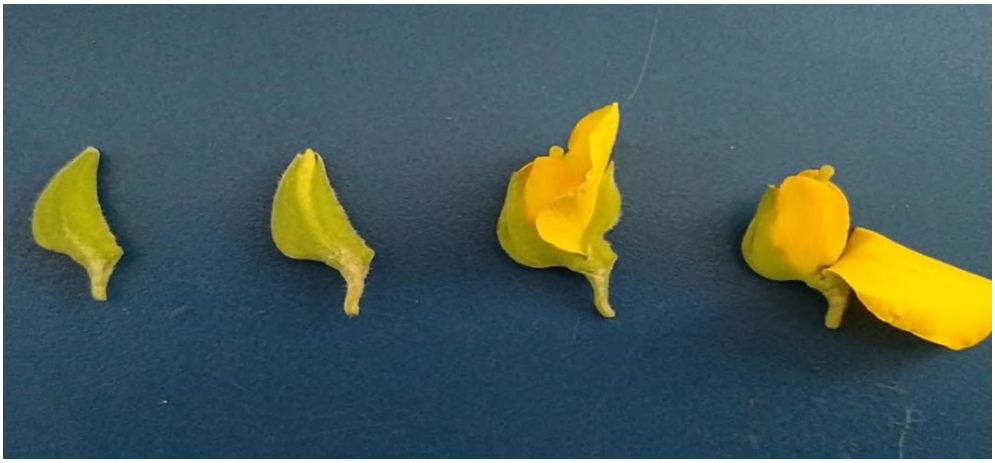

Fig.1.Various stages of flower opening in Crotalaria juncea

1) Fully mature unopened buds 2) Initiation of emergence of standard petal
3) Fully emerged standard petal 4) Fully opened flowers

Fig.2. Receptive stigma generating bubbles

143.6 cm and K12 Yellow being the tallest at 184.4 cm. The height of the first branching ranged from 83.6 cm in SUIN 053 to 133.4 cm in K 12 Yellow. Thirty days after sowing, the genotypes were observed to have about 2 to 5 secondary branches. All the genotypes showed determinate growing habits and had about 7 to 9 grooves on their stem. All the leaves were green in colour, lanceolate in shape and alternate in the arrangement. The leaf length varied from 9.84 (CO 1) to 14.5 cm (K 12 Yellow), while leaf breadth ranged from 1.9 (CO 1) to 3.3 cm (K 12 Yellow). The stem girth ranged from 1.5 cm in CO 1 to 2.2 cm in K 12 Yellow. K 12 Yellow and CO 1 can be differentiated based on the stem girth as K 12 Yellow was the thickest and CO 1 was the thinnest.

The time of first flowering varied between 27 days in CO 1 to 40 days in JRT 610. Time to 50% flowering ranged from 31 days in CO 1 to 45 days in JRT 610. All the genotypes were observed to be lightly stipulated. Plant growth habit was not a parameter for genotype identification as all the genotypes were determinate in habit. The tallest genotype K 12 Yellow had the thickest stem. Similar observations were recorded by (Cook and White, 1996 and Kumar *et al.,* 2008). The characters *viz.,* the number of stem grooves and stipules were almost similar in all the genotypes. K 12 Yellow had the maximum leaf length and leaf breadth (14.5 and 3.3 cm, respectively), while CO1 had the minimum (9.84 and 1.9 cm, respectively) reading for both the characters.

The flower colour of standard petals of all the genotypes was golden yellow and anthers were yellow with white filaments. All the genotypes showed anther dimorphism and had heart and globose shaped anthers. A similar finding about anther dimorphism was reported by Etcheverry (2000). Dark patternings-streaks on petals were visible in SUIN 037 and CO 1, while other genotypes did not have patternings. The number of flowers per inflorescence varied between 10 in SUIN 053 to 17 in CO 1. The peduncle length was the shortest in SUIN 037(25.4 cm) while CO 1 had the longest length (39.6 cm).

Table 1. Biometrical characteristics of selected six genotypes of sunnhemp

S.No.	Character	Genotypes						Mean	CD
		SUIN 053	SUIN 037	K 12 Yellow	JRT 610	CO 1	SN 23		
1	Plant height (cm)	143.6	164.4	186.4	160.4	158.4	163.2	162.73	6.8
2	Height of first branching (cm)	83.6	104.4	133.4	117.4	92.2	110.3	106.87	7.4
3	Number of secondary branches	4	5	5	3	4	2	3.83	0.8
4	Stem grooves	7	9	9	8	9	9	8	0.9
5	Stem girth	1.7	1.7	2.2	1.9	1.5	1.8	1.8	0.4
6	Leaf length (cm)	11.6	13	14.5	10	9.84	10.33	11.55	2.6
7	Leaf breadth (cm)	2.1	2.2	3.3	2.4	1.9	2.2	2.35	0.3
8	Time of first flowering (days)	30	35	38	40	27	35	34.17	6.8
9	Time of 50% flowering (days)	35	40	43	45	31	40	39	5.3
10	Number of flowers per inflorescence	10	11	13	11	17	12	12.33	2.4
11	Peduncle length (cm)	25.4	37.2	37.2	29.94	39.6	33.2	33.75	4.2
12	Number of inflorescence per plant	5	4	3	5	4	4	4.17	1.1
13	Number of pods per inflorescence	28.8	32.4	25.2	32.4	36.8	34.7	31.72	7.6
14	Seeds per pod	11.4	10.2	6.5	9.3	13.2	8.9	9.92	2.4
15	Seed yield/ plant (g)	76.4	64.6	54.5	48.6	63.7	69.7	62.92	10.3
16	Seed index	4.04	3.86	3.80	3.94	2.72	3.43	3.56	0.7

Table 2. Qualitative characteristics of selected six genotypes of sunnhemp

S.No.	Character	Genotypes					
		SUIN 053	SUIN 037	K 12 Yellow	JRT 610	CO 1	SN 23
1	Presence or absence of stipulus	Lightly stipuled	Lightly stipuled	Lightly stipuled	Lightly stipuled	Lightly stipuled	Lightly stipuled
2	Plant growth habit	Determinate	Determinate	Determinate	Determinate	Determinate	Determinate
3	Leaf shape	Lanceolate	Lanceolate	Lanceolate	Lanceolate	Lanceolate	Lanceolate
4	Colour of foliage	Green	Green	Green	Green	Green	Green
5	Leaf arrangement	Alternate	Alternate	Alternate	Alternate	Alternate	Alternate
6	Colour of standard petal	Golden Yellow	Golden Yellow	Golden Yellow	Golden Yellow	Golden Yellow	Golden Yellow
7	Petal patternings	No	Yes	No	No	Yes	No
8	Colour of filament	White	White	White	White	White	White
9	Colour of anther	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
10	Shape of dimorphic anthers	Heart and Globose	Heart and Globose	Heart and Globose	Heart and Globose	Heart and Globose	Heart and Globose
11	Immature pod colour	Green	Green	Green	Green	Green	Green
12	Mature pod colour	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
13	Pod shape	Inflated	Inflated	Inflated	Highly inflated	Inflated	Inflated
14	Pod pubescence	Medium	Medium	High	Medium	Medium	Medium
15	Pod tip	Blunt	Blunt	Blunt	Blunt	Blunt	Blunt
16	Seed placentation	Marginal	Marginal	Marginal	Marginal	Marginal	Marginal
17	Seed colour	Slate	Slate	Yellow	Slate	Slate	Slate
18	Seed texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
19	Seed size	Medium	Medium	Large	Larger	Small and flat	Medium
20	Seed shape	Kidney	Kidney	Kidney	Kidney	Kidney	Kidney

Colour of standard petal, anthers, anther filaments colour and anther dimorphism were similar for all the genotypes and thus it cannot be used for varietal differentiation. Petal patterning was unique for SUIN 037 and CO 1 and thus patterning attributes can be used for recognition and characterization of the genotypes under study **(Fig.3).** CO 1 and SUIN 053 genotypes can be differentiated by the stark contrast in length of the peduncle.

In all the genotypes, the immature pods were green in colour while the matured pods were brownish yellow in colour. All the genotypes had moderate pod pubescence with the blunt end. All genotypes had marginal placentation and the number of seeds per pod varied from 6 to 7 in K 12 Yellow to 12 to 14 in CO 1. Seeds of all the genotypes except K12 Yellow had slate (blackish-grey) colour, while K 12 Yellow had yellow coloured seeds. The seeds of all the genotypes were smooth in texture and were kidney shaped. The seed size showed considerable variation. K 12 Yellow had large sized seeds, while CO 1 had small and flat seeds, while all other genotypes had medium sized seeds. The seed index (weight of 100 seeds in grams) varied from 2.72 (CO 1) to 4.04 g (SUIN 053) and seed yield per plant varied from 48.6 (JRT 610) to 76.4 g (SUIN 053).

The various characters *viz.,* seed colour, shape, pubescence, pod tip, seed placentation, seed texture and seed shape were comparable in all genotypes and did not show much variation. The seed colour was a major identification feature in which K 12 Yellow produced yellow seeds, while all the others produced slate coloured seeds. The seed shape varied within the genotypes and K 12 Yellow had the largest seeds while CO 1 had the smallest. The seed index was the highest for SUIN 053 (4.04 g), while CO 1 (2.72 g) had the lowest seed index. The seed yield per plant was the highest in SUIN 053 (76.4 g) while it was the lowest in JRT 610 (48.6 g).

Filament length of heart and globose shaped anthers and length of style were recorded at four different stages of flowering (**Table 3**). The length of filaments of globose shaped anthers was more or less the same in all the stages of flowering, while the filaments of heart shaped anthers were shorter than the filaments of globose shaped anthers in unopened buds, but once the standard petal had initiated, the filament of heart shaped anthers grew faster and overshot the filaments of globose anthers. Nirmalaruban *et. al.* (2020) reported similar findings in *Crotalaria juncea* .

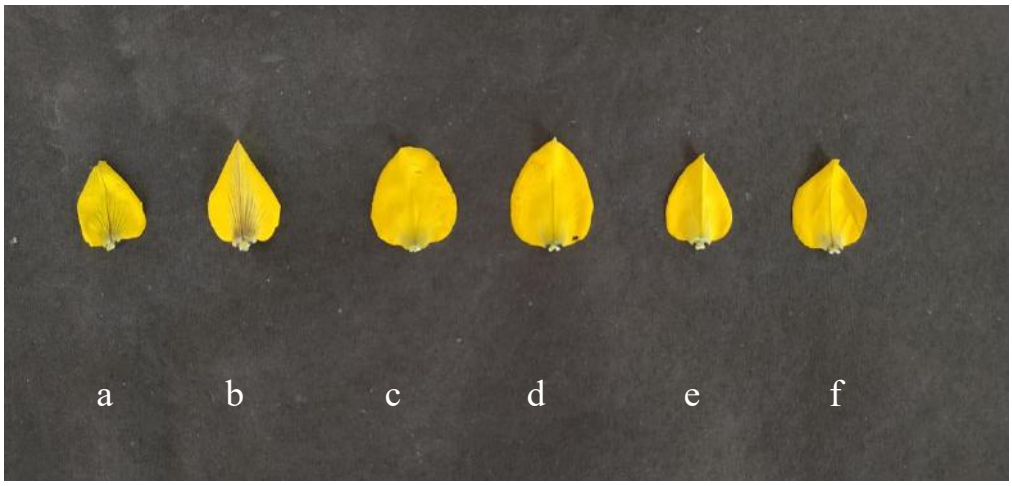

Fig.3. Standard petal patternings on various genotypes

a)CO 1 b) SUIN 037 C) K 12 Yellow d) JRT 610 e) SUIN 053 f) SN 23

Table 3. Variation in filament and style lengths and stigma receptivity at various stages of flower development in selected six genotypes of sunnhemp

S.No.	Genotype	Flower stage	Filament length of heart shaped anthers (mm)	Filament length of globose shaped anthers (mm)	Style length (mm)	Stigma receptivity
1	SUIN 053	Fully mature unopened buds	8	9	14	Low
		Initiation of emergence of standard petal	11	9	15	Low
		Fully emerged standard petal	14	10	17	Medium
		Completely opened flowers	15	10	17	High
2	SUIN 37	Fully mature unopened buds	7	8	14	Low
		Initiation of emergence of standard petal	7	9	17	Medium
		Fully emerged standard petal	10	10	17	High
		Completely opened flowers	14	11	17	High
3	K12 Yellow	Fully mature unopened buds	9	10	17	Very low
		Initiation of emergence of standard petal	11	10	18	Very low
		Fully emerged standard petal	13	12	19	Medium
		Completely opened flowers	16	12	19	High
4	JRT 610	Fully mature unopened buds	8	9	14	Low
		Initiation of emergence of standard petal	10	10	15	High
		Fully emerged standard petal	11	10	15	High
		Completely opened flowers	12	10	15	Very high
5	CO 1	Fully mature unopened buds	8	8	14	Low
		Initiation of emergence of standard petal	10	9	15	Medium
		Fully emerged standard petal	11	9	16	High
		Completely opened flowers	13	9	16	Very high
6	SN 23	Fully mature unopened buds	8	10	14	Low
		Initiation of emergence of standard petal	10	11	14	Medium
		Fully emerged standard petal	12	11	15	Medium
		Completely opened flowers	13	11	15	Very High

During the initial phases of growth, the stigma was non receptive while it became receptive after the dehiscence of globose shaped pollen grains and continued to be receptive up to the dehiscence of heart shaped pollen grains. Although the anthesis was between 12 pm to 4 pm, the globose shaped pollen grains were found to be released 24 hours prior to anthesis owing to the protandrous nature of *C juncea,* while the heart shaped pollen grains were released 24 hours after flower opening (Etcheverry *et al.*, 2003). The stigmas became receptive after 12 pm, in between the time period after the dehiscence of the globose shaped anthers and before the dehiscence of the heart shaped anthers. The stigma remained receptive for one day and maximum receptivity was shown when the flowers were completely opened.

In vitro pollen viability, germination and pollen tube growth of selected five sunnhemp genotypes and their *in vivo* pollen tube growth in the pistil of SN 23 sunnhemp genotype are given in **Table 4**. Results of *in vitro* viability test of pollen grains of five genotypes in artificial medium showed variation. SUIN 37 showed the highest viability of 93.15 per cent, while JRT 610 showed the least viability of 83.34 per cent. The pollen viability of globose and heart shaped anthers of each of the genotypes was documented and found to differ negligibly.

The pollen grains showed maximum viability when the standard petal fully emerged. *In vitro* pollen germination was recorded and it has been observed that all the viable pollen grains did not germinate and about 8-14 per cent

Table 4. *In vitro* pollen viability, germination and pollen tube growth of selected five sunnhemp genotypes and their *in vivo* pollen tube growth in the pistil of SN 23 sunnhemp genotypes

S. No	Genotype	Pollen viability %		Per cent of pollen germination	Per cent of pollen with normal pollen tube growth	Total length of style of SN 23 (mm)	Length of pollen tube (mm) in vivo after different hours of cross pollination					Length of pollen tube (mm) in vitro in artificial medium		
		Heart shaped anthers	Globose shaped anthers				2 hours	4 hours	6 hours	8 hours	12 hours	2 hours	4 hours	6 hours
1	SUIN 053	92.47	90.44	82.19	64.47	14.83	2.81	6.39	10.68	13.48	14.83	2.94	4.03	13.24
2	SUIN 037	91.96	88.32	83.38	70.89	14.83	2.83	6.78	11.94	14.83	14.83	3.06	4.26	14.79
3	K 12 Yellow	88.64	90.39	78.13	60.59	14.83	2.46	5.92	9.88	12.14	12.14	2.87	4.19	13.21
4	JRT 610	90.27	88.74	77.22	59.42	14.83	2.43	5.46	9.37	12.09	12.09	1.84	6.36	13.14
5	CO 1	91.82	88.20	80.72	63.27	14.83	2.52	6.29	10.03	12.61	12.61	2.24	4.67	11.10

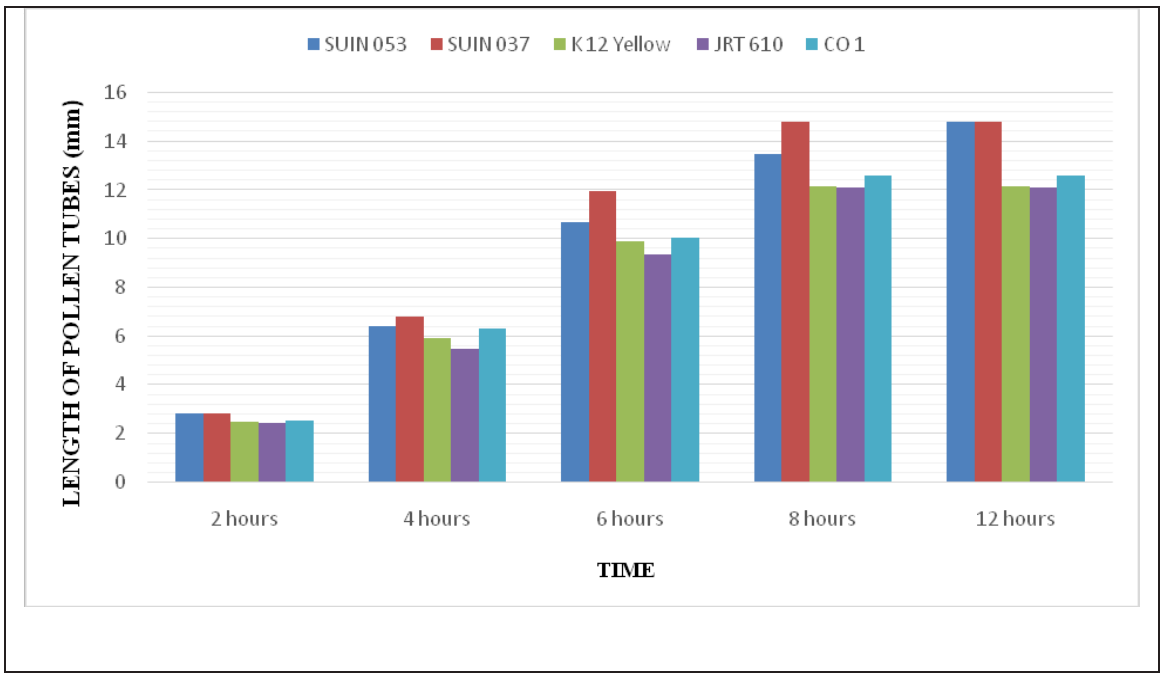

Fig. 4. Pollen tube length of five sunnhemp genotypes in the pistil of SN 23 genotype

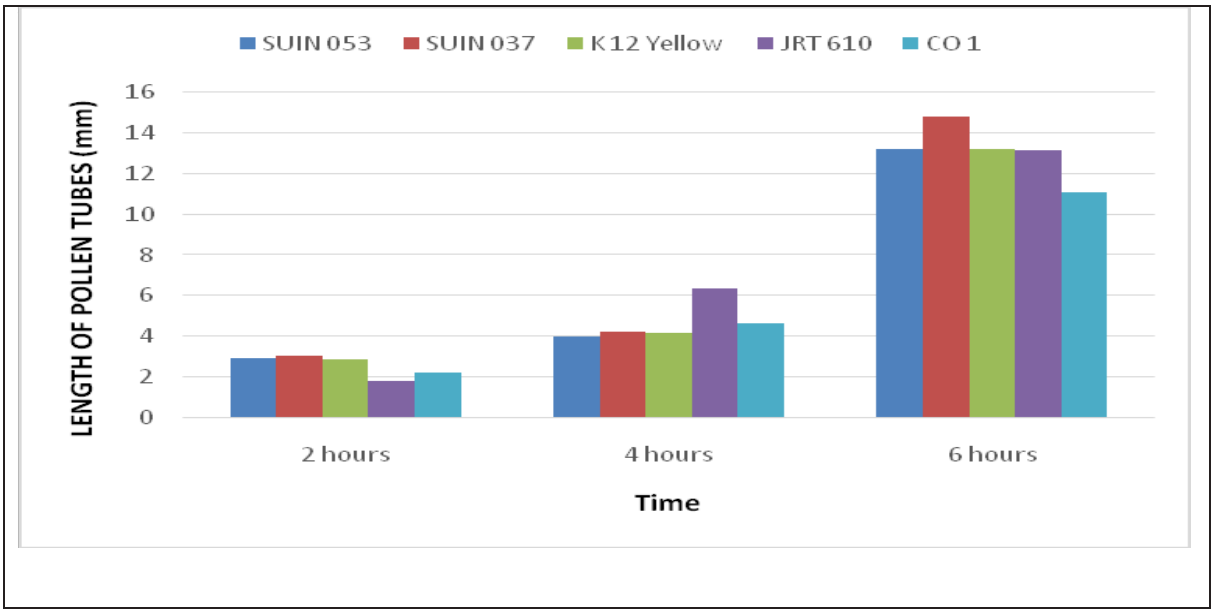

Fig. 5. Pollen tube length of five sunnhemp genotypes in artificial media under *in vitro* conditions

drop in the count was observed. Out of the germinated pollen grains, about 77 to 81 per cent showed normal pollen tube growth. There existed a direct relationship between pollen viability and the number of pollens with normal pollen tubes.

Under *in vivo* conditions, the time taken by the crossed pollen tubes of selected genotypes to reach the base of the style of genotype SN 23 was recorded **(Fig. 4).** The time taken by the pollen tube to reach the ovary varied with the male genotype. It was evident from the data that the pollen tubes of the genotype SUIN 037 showed better growth than the other genotypes and was the fastest to reach the base of SN 23's style leading to the ovary in 8 hours *in vivo*. Followed by SUIN 037, the genotype SUIN 053 reached the ovary 12 hours after pollination. The other three genotypes stopped their pollen tube growth at the base of the style and did not show further growth after 8 hours. Under *in vitro* conditions among the pollen tubes of five genotypes, SUIN 037 showed faster growth than the other four sunnhemp genotypes **(Fig. 5).**

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