J. Assam Sc. Soc. Vo. 56, No.2
December 2015; PP. 39-48

ISSN 0587-1921

MULTILOCATIONAL TRIAL FOR GROWTH, YIELD ATTRIBUTING CHARACTERS AND SCANNING ELECTRON MICROSCOPIC STUDY OF POLLEN GRAINS OF UNDER-UTILIZED CEREAL *Coix-lacryma-jobi* L. var Ma Yuen Stapf.

Chandra Kanta Saikia[1], Zalio Seyie[2] and *Gautam K. Handique[3]

[1] Institute of Science and Technology, GauhatiUnivesity

[2] Department of Biotechnology, GauhatiUnivesity

[3] Department of Botany, Nalbari College

**Corresponding author:* gautamkhandique@rediffmail.com

ABSTRACT

A study was conducted for growth and yield attributing parameters of the under-utilized cereal Job's tear (*Coix-lacryma-jobi* var Ma Yuen) under three different geographical and climatic conditions. Best performance was recorded under high altitude condition of Khonoma (1524m) followed by mid altitude condition of Medziphema(305m)and low altitude valley condition of Dimapur. Despite the difference in performance the promising aspect is that this lesser known crop has significant resilence and adaptability under

diverse geographical conditions. Palynological study by scanning electron microscope shows that it has all the typical characteristics of cereal crop.

Key words: Under-utilised crop, *Coix-lacryma-jobi*, scanning electron microscopy, pollen grain

INTRODUCTION

Underutilized and lesser known crops are getting increasing importance in recent time for a number of reasons. There is a growing realization that conventional crops will be unable to meet the growing demand for food, as food production globally and nationally are by and large stagnating since the beginning of 21st century. The food scarcity of 2008 was transient but it served as alarm bell for future.Under-utilized crops, which are adapted to stressed environment and provide food and nutritional cover to 10% population inhabiting remote, tribal and backward areas offer scope for diversification of agriculture (Phogot et al. 2006). Therefore study of such crop species in terms of nutritional value, genetic diversity and characterization is a priority, but changing land use pattern , climate change and other such factors have led to the depletion of such genetic resources (Swaminathan 2011, Hedge et al. 2015) which is a matter of concern. In the backdrop of such gloomy situation one promising way is to evaluate and utilize lesser known food crops. It is known that out of 80,000 plant species known to be edible for human, not more than that 350 are put to major use. Again more than 90% of global food production come from about 30 crop species (Bhag Mal 1990). These statistics imply that through scientific scrutiny it is possible to make addition to the food basket.

Coix-lacryma-jobi var.Ma Yuen (Job's tear) is an ancient crop of limited cultivation by many ethnic tribes of South East Asia, China and India(Venkateswarlu and Changanti 1973). In India its cultivation is prominent in hilly terrain of Nagaland with remarkable diversity of germplasm and local tribal refer it as "Paddy substitute" (Handique et al. 1986). Phylogenetically it is however, closer to maize and considered as minor cereal. In remote areas with poor road communication with hilly terrain it is still cultivated in limited pockets. There is no information regarding whether this crop can be grown in flat land of plain areas. Understanding adaptive property of a crop species is pre-requisite before recommending for large scale cultivation in non-traditional areas. However, nutritional quality analysis shows that it is superior to best cereals with protein content in the range of 15% to 18%, and lipid content 3.03% to 5.50%. For carbohydrate, crude fiber and total mineral it is comparable to paddy (Handique 1997).Available information therefore necessitates rethinking about this neglected crop. Because while production and productivity is related to food security; nutritional quality is related to nutritional security. In the context of overall food scenario nutritional security is as important as food security(Swaminathan 2011)

The present study was based on the collection of germplasm of the crop from various parts of Nagaland to evaluate their field performance in different geographical and climatic conditions. The electron microscopy of pollen grains was also done to assess how much it corroborate with earlier reports that the crop is mainly self-pollinating type (Handique 1997).

MATERIALS AND METHODS

Germplasm collection and multilocational field trial

15 land races of *Coix-lacryma-jobi* var. Ma Yuen (soft shelled job's tear) were collected from different districts of Nagaland. Highest collection of four was made from Kohima district. Seeds were collected from farmer's fields including jhum field, household through a number of field visit. As per conventional practice the land races were primarily demarcated on the basis of seed morphology and seed colouration and designated as KS-1,2,3 etc. For multi-location field trial 3 localities were selected based on the differences in geographical and climatic factors. These were Khonoma (near Kohima, state capital) categorized as high altitude (1524m) with hilly terrain (2) Medziphema, mid altitude (305m) with hilly terrain /foothill (3) Dimapur valley with plain land. The basic weather parameters for the cultivation period are as follow-

Table 1. Meteorological data of the three location

Month	Place	Temperature(^{0}C)		Average Rainfall(mm)	Relative humidity(%)
		Max.	Min.		
June	Khonoma	28.20	16.00	302.30	69.50
	Medziphema	31.05	23.99	297.90	67.13
	Dimapur	31.24	24.89	288.92	68.12
July	Khonoma	28.20	16.50	302.22	68.20
	Medziphema	31.37	23.64	223.80	63.09
	Dimapur	32.97	25.10	234.81	64.21
August	Khonoma	27.15	16.85	301.50	67.50
	Medziphema	31.65	24.86	207.60	67.86
	Dimapur	33.11	25.63	211.58	68.92

September	Khonoma	26.75	15.90	290.50	67.21
	Medziphema	30.79	22.91	286.60	66.86
	Dimapur	32.69	23.71	298.72	67.96
October	Khonoma	24.40	15.20	285.21	62.50
	Medziphema	28.62	20.89	171.30	64.10
	Dimapur	29.86	21.92	178.89	65.28
November	Khonoma	23.50	12.10	0.00	40.21
	Medziphema	26.72	14.11	0.00	47.20
	Dimapur	23.50	15.54	0.00	48.36

Field experiment layout was made as per randomized block design. Plot size was 3×2.5meters with 3 rows per plot. Only farmyard manure was applied at the rate 3kg per plot. Except weeding at the seedling stage, no other treatment was given and the plants were allowed to grow under natural rain fed conditions. Seeds were sown in the 2nd week of April and harvested in the first week of November. Data were recorded from 10 plants randomly selected from each plot. The parameters taken were-height at maturity, number of tillers, biomass kg/15sq.m, seed yield per plant and weight of 100seeds. The data were processed by analysis of variance.

Table: 2 Growth characteristics and yield attributing characters of the *Coix* cultivars grown in Dimapur(Low altitude)

Cultivar	**Height at maturity(m)**	**No. of tillers**	**Biomass kg/15^{2}m**	**Seed yield per plant (g)**	**Weight of 100 seed (g)**
KS1	1.45	6.0	10.9	152.4	11.05
KS2	1.65	6.2	12.6	168.3	14.00
KS3	1.72	8.1	17.8	181.7	9.05
KS4	1.60	9.3	16.5	140.0	14.05

KS5	1.77	5.3	14.1	178.6	16.82
KS6	1.48	7.0	11.7	104.2	9.00
KS7	1.61	7.2	15.0	120.1	9.22
KS8	1.40	9.1	13.6	130.5	10.26
KS9	1.51	5.4	11.2	106.7	17.01
KS10	1.40	10.1	14.1	141.3	12.00
KS11	1.70	6.3	15.2	154.6	12.05
KS12	1.42	9.2	13.3	153.5	8.15
KS13	1.48	5.4	13.0	117.2	17.16
KS14	1.40	7.1	14.9	190.3	14.15
KS15	1.58	10.0	16.6	178.4	15.00
CD at 5%-	0.529	0.982	1.913	2.510	2.007
CD at 1%-	0.713	1.324	2.579	3.383	2.706

Table3: Growth characteristics and yield attributing characters of the *Coix* cultivars grown inMedziphema(Mid Altitude).

Cultivar	**Height at maturity(m)**	**No. of tillers**	**Biomass kg/15^{2}m**	**Seed yield per plant (g)**	**Weight of 100 seed (g)**
KS1	1.60	7.3	12.4	165.0	11.93
KS2	1.77	6.5	14.8	180.8	14.15
KS3	1.80	10.1	20.6	208.6	9.82
KS4	1.65	10.2	18.8	148.2	14.6
KS5	1.90	6.1	16.7	190.7	17.5
KS6	1.55	9.0	12.8	112.0	9.6

KS7	1.70	8.4	16.8	129.8	9.8
KS8	1.45	10.0	15.5	138.4	10.6
KS9	1.60	5.5	12.8	115.6	17.58
5K8	1.48	12.0	16.0	150.8	7.46
KS11	1.72	7.3	16.6	170.7	12.5
KS12	1.40	11.1	15.1	128.4	8.96
KS13	1.55	5.4	13.6	120.6	18.37
KS14	1.85	8.1	17.0	202.4	14.45
KS15	1.66	12.0	19.8	190.5	15.28
CD at 5%-	0.562	0.466	1.977	2.330	1.797
CD at 1%-	0.756	0.629	2.666	3.141	2.422

Table 4: Growth characteristics and yield attributing characters of the *Coix* cultivars grown in Khonoma (High altitude)

Cultivar	**Height at maturity(m)**	**No. of tillers**	**Biomass kg/15^{2}m**	**Seed yield per plant (g)**	**Weight of 100 seed (g)**
KS1	1.75	9.0	14.2	170.0	11.64
KS2	1.70	8.1	16.1	190.9	14.50
KS3	1.95	10.2	22.0	215.2	10.00
KS4	1.70	12.4	20.4	153.0	15.05
KS5	1.84	7.3	17.0	193.6	18.10
KS6	1.72	10.0	14.3	120.4	10.00
KS7	1.78	8.3	17.5	123.5	10.00
KS8	1.53	12.1	19..2	142.7	11.30

KS9	1.58	7.2	12.0	118.3	17.05
KS10	1.55	14.1	20.6	158.0	7.54
KS11	1.75	7.4	17.0	171.5	12.50
KS12	1.52	10.3	18.9	130.8	8.75
KS13	1.60	7.1	15.6	118.8	18.05
KS14	1.78	7.4	15..1	197.6	14.15
KS15	1.72	12.3	21.3	195.6	15.34
CD at 5%-	0.070	1.063	1.939	2.443	1.721
CD at 1%-	0.094	1.434	2.614	3.293	2.320

Study of pollen grains by scanning electron microscope

Freshly collected pollen grains were allowed to dry overnight at room temperature before electron microscopy. Subsequently the pollen grains were embedded on a metals stub coated with epoxy resin. The samples were sputter coated with gold using ion sputter JFC-1100 having a gold plated cathode. The pollen grains were examined with scanning electron microscope model JSM-35FCF (JEOL) operated at 15kv under 720X and 2000X magnification. The facilities available at Regional Sophisticated Instrumentation Center, NEHU Shillong were availed for the work.

Results and discussion

Considerable variations were observed for all the growth parameters. Both cultivar to cultivar variation and location to location variations were evident; the former being more prominent with respect to height at maturity. Best performance was observed at high altitude location of Khonoma; where the range of variation for cultivars were from 1.52m in KS-12 to 1.95m in KS-3 which was statistically significant. The corresponding figure for mid altitude location of Medziphema was 1.40m(KS-12) to 1.90m(KS-5) and same for valley area of Dimapur was 1.40m (in KS-8, KS-10 and KS-14) to 1.75 in (KS-5). The number of tiller exhibited cultivar to cultivar variation as well as location to location variation with a trend similar to height. Under Khonoma condition tiller numbers were comparatively higher in the range of 7 to 14. In mid altitude condition of Medziphema the same declined a little; the range of variation was 5 to 12. For valley condition of

Dimapur,tillering was comparable to that of Medziphema. Biomass per unit area was highest under high altitude condition and varied in the range of 14.0 to 22.0 kg/15sq.m. The cultivar to cultivar variations were significant. Under valley condition of Dimapur biomass yield were comparativly low, in the range of 10.9 to 17.8kg/15sq.m. For mid altitude condition of Medziphema, biomass yields were intermediate to both in the range of 12.4 to 20.6kg/15sq.m.

For yield attributing characters also there were significant variation for cultivar to cultivar as well as locations. Under high altitude condition of Khonoma significant cultivar to cultivar variation was observed in the range of 123.5g/plant to 215.2g/plant. The corresponding figures for mid altitude condition of Medziphema was 112.0g/plant to208.6g/plant. Under valley condition of Dimapur the values dropped further to 107.2g/ plant to 183.2g/plant; which is the lowest among the three locations. Variation in 100 seed weight also exhibited a similar pattern (Fig. 1). Under high altitude condition of Khonoma the lowest value 8.75g was observed for KS-12 while highest value was recorded for KS-5 with 18.10g. which was more than double of the lowest value. Unlike all other parameters under mid altitude condition of Medziphema 100g seed weight did not decline but remained same with little increase;the range of variation being 8.96g to 18.37g. Even in valley condition of Dimapur also 100seed weight remained nearly unchanged in the range of 8.15 to 17.16g.

Scanning electron microscopy (Fig. 2) revealed that the pollen grain were circular, thin walled with smooth and little wavy surface. Pollen grains from several cultivars taken at random revealed the same feature. Only one germ pore was noticed per pollen grain. Pollen diameter varied from 63 to 66µ. Thus, the pollen grains resembles all the features of typical air-borne pollen of Graminaceous plant. Freshly collected pollen grains at the time of anthesis (9AM) showed 92 to 95% viability as determined by acetocarmine staining method. However, 6 hours after anthesis if dropped to 80-82%. After 24 hours, viability was found to be lost. The pollen grains did not respond to wide range of sucrose solution. There was also no response to 1% TTC(2,3,5-triphenyl tetrazolium chloride) + 5% sucrose as well as aqueous extract of stigma.

Multi-locational field trial is a reliable and widely accepted technique to assess the suitability of a cultivar or a crop species in a location with particular geographical and climatic factor. The famous Basmati rice varieties grow well and hence cultivated on commercial scale only in some pockets of North India, particularly Dehradun valley. Saha et al. (1979) demonstrated the utility of multilocational trial to show that Tripura is far more suitable than other locations Jorhat (Assam) and Nagaland for citronella

Figure 1: Seeds of different land races of *Coix lacryma-jobi* showing wide variation in seedmorphology and coulour

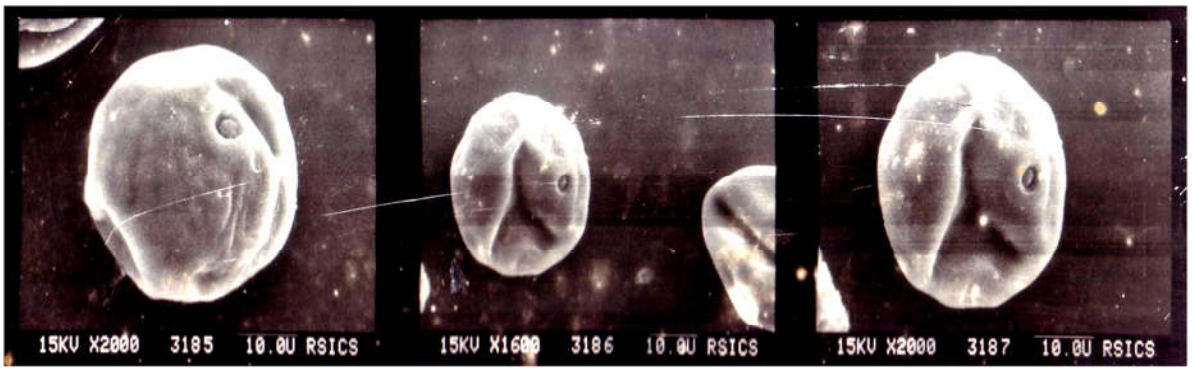

Figure 2: Scanning electron micrograph of the pollen grain of *Coix lacryma-jobi* with 600x magnification (middle) and 2000x magnification (left and right).The single germ pore is prominently visible

A

B

C

D

Figure 3: *Coix lacryma - jobi* plantation at different growth stages

cultivation. Similar field trial revealed that exotic medicinal plant *Dioscorea floribunda* of Central America is adaptive to the climatic condition of Assam (Singh et al.1978) apparently due to similarity in climatic and adaptive factors. *Coix-lacryma-jobi* occurs in both wild and cultivated forms; wild types belong to the varities Stenocarpa and moniliform and are characterized by slippery surface and with hard shell. The other type is known as var. Ma-Yuen. It has soft shell that can be dehusked and edible (Arora, 1977). *Coix-lacryma-jobi* has two sub species. *Stenocarpa*, characterized by hard slippery surface which is non-edible. Typically it grows as weed in marshy land and plain lands like river valley (Fig. 3). The other has grains with soft shell that can be dehusked and edible. This sub species is Ma-Yuen; but donot grow in water logged condition or marshy land (Handique et al. 1986). The present study show that land races of Ma-Yuen has considerable adaptability and resilence. While in traditional cultivation areas of Khonoma (high altitude areas) and Medziphema (mid altitude areas) it grows well; in non -traditional areas like Dimapur (plain land/river valley) also it performs reasonably well although its growth and yield are lower than that of traditional areas. The study further reveal that while environmental factors significantly influence growth parameters; yield attributing characters particularly 100 seed weight remain least affected.

SEM study reveals valuable information about pollination behaviour. There are reports that *Coix-lacryma-jobi* is basically self-pollinating crop although cross pollination may occur occasionally (Handique 1997). Smooth surface, thin cell wall with single germ pore are indicative of air borne self-pollination similar to that maize. Short duration of pollen viability is another feature which is comparable to that of other known self-pollinating crops like paddy maize etc. and in conformity with earlier reports(Handique 1997). The pollen grains are nearly two times bigger than that of paddy pollen grain.

Overall, the multi-locational trial reveal that both high and mid altitude location with hilly terrain or sloppy lands are ideal for its cultivation. In terms of overall suitability of geographical and climatic requirement; the order of locations are Khonoma (high altitude)>Medziphema (mid altitude)>Dimapur(plain/valley area). The present study shows that this crop is adaptive and resilient enough to be cultivated in non-traditional areas and could be suggested to introduce the crop for large scale cultivation.

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