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Profiling mahua (*Bassia latifolia*) accessions for flower characters and nutraceutical attributes under north Indian conditions

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ABSTRACT

Mahua (*Bassia latifolia* Roxb.) is a characteristic spontaneous crop species found scattered throughout the tropics and subtropics of the Indian subcontinent. Categorized as an underutilized fruit crop, there is considerable scope to evaluate and characterize the available diverse germplasm in natural stands for promoting its utilization on commercial scale. Keeping the importance of variability in view, 24 accessions collected from different diversity hot spots of the country during 2006-2010 were asexually propagated on seedling rootstocks, planted in the field gene bank and evaluated for flower biology, physicochemical and nutraceutical parameters of the fruit. Approach herkogamy demonstrated by spatial separation of sex organs, existed in the species as an out-crossing mechanism allowing gene flow and genetic introgression in natural stands that contributed to the prevailing diversity. The pollen was 45-55 µm in size, anemophily coexisting with geitonogamy appeared to be the mechanism of pollen transfer. Thus, the flower biology demonstrated floral attributes that accounted for cross pollination resulting in genetic introgression and generation of appreciable diversity in natural stands, emphasizing needs of its characterization for profitable utilization. Based on the attributes, CISH M-4, CISH M-3 and CISH M-8 showed promise in respect of different fruit quality parameters. CISH M-4 outscored others with regard to juice content (65.42 %), pomace (37.94), TSS (26.40 ^{0}Brix), Total sugar (23.497 %), Vit C content (64.163 mg/100g) and antioxidant value (44.483 mg AEAC/g). Lack of improved cultivars for commercial utilization, can thus be attended by focused evaluation of mahua germplasm accessions for the fruit quality parameters and their exploitation in further crop improvement programmes.

Key words: *Bassia latifolia*, Flower biology, Herkogamy, Proximate composition

Mahua (*Bassia latifolia* Roxb.), member of Sapotaceae and native to the Indian subcontinent is found endemic to the forests of North Indian plains. Globally, the species is found distributed in India, Sri Lanka and possibly, Myanmar. In India, the species is mainly found distributed across the peninsular region in the states of Chhattisgarh, Jharkhand, Uttar Pradesh, Bihar, Madhya Pradesh, Kerala, Gujarat and Odisha (Chatterjee and Pakrashi 2003; Patel and Naik 2010). The two predominant species found in India are *Madhuca indica* Gmel. (Syn. *Bassia latifolia* Roxb.), a deciduous species ubiquitous to the northern parts and *Madhuca longifolia* Macb. (Syn. *Bassia longifolia* Koeing.), an evergreen species common to the southern parts of the country (Prasad and Prasad 1991). Mahua flowers are rich source of sugars, protein, vitamin and minerals, and the wood is suitable for timber and making paper (The Wealth of India 1962). Next to sugarcane, mahua flowers are the most important source of raw material for fermentation and production of alcohol and vinegar and also as feed for the livestock. Oil-bearing seeds of the species are often the main economic product as seeds contain 20-50% oil which can be used in soap industry and oil seed cake as manure (Daniel and Hegde 2007). Due to cross pollination and predomination of seed propagation, there exists enough genetic diversity providing opportunity to locate elite and distinct accessions from the areas of diversity hotspots. Wide variations were reported earlier for sweetness, acidity, size and shape of inflorescence and canopy configurations under UP conditions (Singh *et al.* 1999). Proper selection of genotypes for traits together with advances in post-harvest handling and value addition protocols can provide for its exploitation in production of industrial alcohol feasible to position it as one of the valuable sources of renewable, low cost energy. This assumes considerable significance in view of the fact that *mahua* oil methyl esters can be used as alternative to biodiesel in India. (Kapilan and Reddy 2008, Ghadge and Raheman 2005). An in-depth variability assessment of available germplasm accessions is therefore crucial through profiling of economic traits,

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including morphological, reproductive phenology and physicochemical attributes for commercial exploitation of this crop. In this background, the present study was undertaken to study flowering and breeding behavior of mahua natural stands under *in situ* conditions, including profiling for nutraceutical attributes.

MATERIALS AND METHODS

The mahua trees found throughout Uttar Pradesh and the adjoining states of Bihar, Madhya Pradesh, Jharkhand, Chhattisgarh and Gujarat were surveyed during 2006-2010 with a view to identify elite germplasm among the existing natural stands. Twenty four promising accessions were identified and multiplied asexually for field GeneBank augmentation at CISH, Lucknow based on initial assessment of physico-chemical attributes. Based on this, ten elite accessions, viz. CISH M-1, CISH M-2, CISH M-3, CISH M-4, CISH M-5, CISH M-6, CISH M-7, CISH M-8, CISH M-9 and CISH M-10 were identified and shortlisted for the study of proximate principles . Flower biology was studied by estimating the total number of flowers produced in fascicles in naturally growing trees from the vicinity of the institute located around Rehmankhera representing largely of dry deciduous forest habitat. The germplasm accessions planted at the institute showed similar flowering attributes to that to fully grown 20 years old road side avenue trees. Mean number of flowers per fascicle and the length of flowers from the base to the tip of corolla and up to the stigmatic tip of the extruded gynoecium were measured to decipher morphological features assisting pollination syndrome in the species. Twenty healthy fascicles from each tree were randomly selected for recording number of flowers (individual and in germplasm) and fruits set per fascicle. The ripe fruits of different shape, size and appearance were sampled randomly for study of detailed proximate composition in different accessions. Total Soluble Solids (^{o}B) were determined by using hand refractometer. Ascorbic acid content of fruits was estimated using 2,6-dichlorophenol indophenol dye method (Ranganna 1986). The total antioxidant activity was estimated in terms of FRAP (Benzie and Strain 1996) while the predominant antioxidant contributing compounds such as total carotenoids (Ranganna 1986), flavonoids (Kim *et al*. 2003) and total phenols (Ranganna 1986) were determined by using UV-visible spectrophotometer.

RESULTS AND DISCUSSION

Reproductive phenology and flower biology

Conforming to its deciduous nature, leaf fall occurred from January to April, with flowers appearing in fascicles having long pedicels (30-35 mm, n =10) during March-April (2009-12) which aligned with its description from China and UK flora (Shu-Kang and Pennnington 1996). A 50 year old tree with full grown canopy produced thousands of flowers within a span of 20 days. The trees produced numerous flowers in dense fascicles at the end of branches borne on long pedicels, with large floral display, supposedly accounting for large amount of pollen removal and cross pollination success (Ishii and Sakai 2001, Kuruvilla 1989). The flowering lasted for more than 3 weeks and was characterized by strong characteristic odour. Flowers were dull brown colored with red tinge, corolla fleshy, juicy, stamens adnate to the corolla; free of one another; two whorled. The calyx was brown, leathery and covered the entire corolla until the flowers opened and the style exserted (Table1 and Fig 1). The corolla was creamy-white, fleshy, ovoid, fused tube with lobed ends, tiny openings at the distal end, functionally responsible for pollen dispersal. Stamens were found arranged in two rows and attached to the corolla tube. The style was long (23.6 mm, n = 10) and thus, even in unopened flowers, the style extruded well beyond the corolla (9.29 mm, n = 5). Negative values for style exsertion in unopened immature buds gave way to positive score for protruding style (-0.24 and 3.79, 6.6, respectively) as well as positive values for occurrence of herkogamy (Table 1 and Fig 1). Pubescence in corolla tube demonstrated its secondary pollen presentation role (Castro *et al*. 2008). Thus closed calyx at the time of stigma exsertion, pubescent corolla tube, ample pollen reward, pollen size and spatial separation of the sex organs at the time of stigma receptivity and anther dehiscence, all evidently are important adaptations for the operation of approach herkogamy in the species studied that perhaps represented increased specialization to ‘anemophily’. Further, this also appears to be significant in view of the observation that it could be a case of less of evolutionary flexibility and exclusion of deleterious mutations which subscribed to the hypothesis that self fertilization is an evolutionary dead-end (Takebajashi and Morrell 2001, Kelly 1997). Abundant pollen production by Mahua forming clouds when the flowers are disturbed has earlier been reported (Kundu 2012), which also endorsed prevalence of anemophilous mechanism. Bats foraging activities and their role in pollination however, could not be ascertained in the present study as reported earlier (Nathan *et al*. 2009).

Table 1 Floral measurements and stigma exsertion during different flowering phenological stages

Flower bud (mm)	Corolla length (mm)	Stamen length (mm)/ Anther state(I/D)	Style length (mm)	Stigmatic exsertion (SE=SL-CL)	Herko-gamy (SL-St.L)
1.73	1.72	0.68(I)	1.48	-0.24	0.8
1.78	1.70	0.73(I)	1.36	-0.34	0.63
1.39	1.30	0.53(I)	1.10	-0.20	0.57
4.20	5.50	2.78(I)	9.29	3.79	6.51
6.30	6.60	4.2(D)	10.2	6.6	6.0

I= Intact, D = Dehisced, SE = Stigmatic exsertion = Stamen length (StL) - Corolla length (CL), Herkogamy = Style length (SL) - Stamen length (StL).

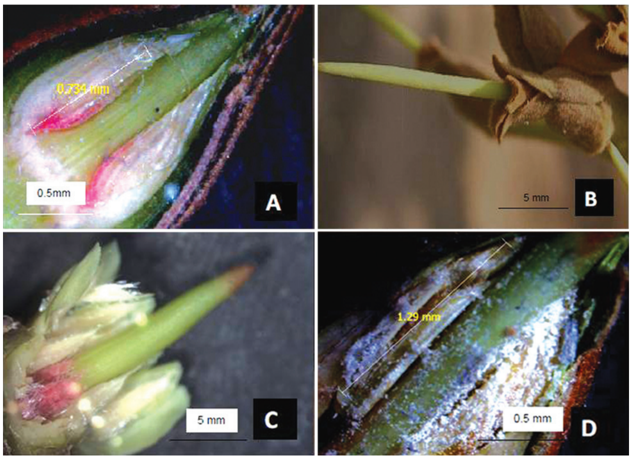

Fig 1 Approach herkogamy demonstrated by spatial separation of sex organs A: LS of flower bud with undehisced anther and adjacent style; B: Style exertion in mature opened flowers; C: Herkogamy: stigma presentation above anther and pubescence for pollen presentation; D: Dehiscences in anthers, with exerted stigma

Variability in yield components and proximate composition

The evaluation of genetic diversity in mahua under subtropical conditions in the present study was primarily based on flower traits (biomass production) and proximate principles. The data pertaining to yield components (flower, juice and pomace contents) and proximate components including phytochemical attributes showed appreciable differences and a high degree of variability for all the characters (Tables 1 and 2). Potential of Mahua flower as a source of nutrition has been explored earlier and documented (Hand Book on Nutritive Value of Indian Foods-ICMR publication), major constituents being sugars, polysaccharides, fibers, proteins, minerals, vitamins, enzymes and organic acids that could be nutraceutically quite useful (Belavady and Balasubramanian 1959). Accordingly, major traits contributing to diversity included flower weight, ranging from 2.207 to 1.187 g, flower length, from 2.18 cm to 1.87 cm and diameter from 1.72 to 1.42 cm with CISH M-4 (3.74 cm^2) recording the largest size. It may also be emphasized here, that early maturing genotypes had relatively higher juice content than the late maturing ones, which is possibly attributed to the prevalence of low temperatures during the early flowering period (March and early April) (Table 2). CISH M-4 outscored all other accessions in respect of juice content (65.42%), pomace (37.94%), TSS (26.40 ^oB) and Vit. C content (64.16 mg/100g). Earlier proximate analysis had reported total sugars content of 40-70% on dry weight basis from different geographical regions (Sarkar and Chatterjee 1983). Similarly, reducing sugar content of 48-57% and non-reducing sugar 3-18% had also been reported elsewhere (Sutaria and Magar 1955, Jayasree *et al*. 1998). Variations in soil and agro-climatic conditions besides maturity times perhaps accounted for wide range of variability in respect of these proximate principles reported among different genotypes in other species (Maiti and Mitra 2002, Sakhyan *et al.* 2004). Mahua juice also contains appreciable nutraceutical principles, the antioxidants, total phenols and carotenoids estimations, which were therefore undertaken to workout the intricate nature of biomass productivity *vis-à-vis* the recovery of active ingredients. Wide variations in the nutraceutical composition among the germplasm accessions were observed (Table 3). The antioxidant values in terms of Ferric Reducing Antioxidant Potential (FRAP) ranged from 9.250 to 44.483 mg AEAC/g in different germplasm accessions. CISH M-4 (44.483 mg AEAC/g) recorded the highest antioxidant activity, while total phenols content ranged from 7.66 to 9.53 mg/g, total carotenoid content however was the highest (18.69 µg/g) in CISH M-1 which otherwise scored poorly in respect of other flower morphological attributes. Based on flower morphological and nutraceutical attributes of different germplasm accessions, it could be inferred that the germplasm accession CISH M-4 could be quite promising as it outscores others for yield components and nutraceutical attribtes. Thus identification of considerable variability in mahua germplasm accessions in the present study, pointed out to

Table 2 Flower morphological attributes and proximate principles in mahua germplasm accessions

Germplasm accession no.	Flower weight (g)	Flower length (cm)	Flower diameter (cm)	Flower size (cm^2)	Juice (%)	Pomace (%)	TSS (^oB)	Total sugar (%)	Reducing sugar (%)	Vitamin C (mg/ 100 g)
CISH M - 1	1.19	1.87	1.46	2.73	44.78	31.05	18.20	20.09	14.92	44.46
CISH M - 2	1.33	1.93	1.51	2.91	59.21	32.77	23.74	21.51	16.19	63.21
CISH M - 3	1.63	2.04	1.69	3.43	62.79	35.20	22.20	22.53	17.73	62.25
CISH M - 4	2.21	2.18	1.72	3.74	65.43	37.94	26.40	23.50	17.53	64.16
CISH M - 5	1.25	1.97	1.42	2.79	55.54	24.17	20.09	20.61	15.38	53.43
CISH M - 6	1.49	2.08	1.49	3.09	56.29	29.93	21.60	20.49	15.20	45.71
CISH M - 7	1.98	2.03	1.62	3.29	63.32	33.77	23.73	21.28	16.52	58.35
CISH M - 8	2.13	2.10	1.64	3.46	63.91	33.37	23.47	22.95	17.46	52.48
CISH M - 9	1.94	2.06	1.67	3.41	61.25	29.30	25.55	22.53	16.62	52.54
CISH M - 10	2.07	1.98	1.60	3.19	60.16	31.46	21.71	22.34	16.15	58.67
SE	0.388	0.089	0.104	0.324	6.016	3.728	2.45	1.153	1	6.976
CD (P=0.05)	0.063	0.120	0.103	0.288	0.908	0.608	2.067	0.648	0.0	1.21

Table 3 Antioxidant assessment in different mahua germplasm accessions

Germplasm accession no.	Antioxidant activity FRAP (mg AEAC/g)	Total phenols (mg tannic acid/g)	Flavonoids (mg catechin/ g)	Total Carote-noids (µg/g)
CISH M - 1	26.570	7.663	0.027	18.687
CISH M - 2	41.353	8.913	0.217	15.193
CISH M - 3	43.557	9.220	0.230	17.777
CISH M - 4	44.483	9.533	0.243	18.520
CISH M - 5	23.607	7.690	0.085	2.253
CISH M - 6	9.250	7.967	0.117	9.553
CISH M - 7	35.457	8.207	0.160	16.790
CISH M - 8	40.427	9.140	0.210	18.250
CISH M - 9	37.707	8.527	0.210	17.250
CISH M - 10	19.403	8.043	0.190	16.420
SE	11.87	0.675	0.07	5.234
CD (P=0.05)	0.653	0.886	0.017	0.921

the scope of further selection for superior types for key economic traits.

The studies demonstrated floral attributes, viz. approach herkogamy that accounted predominantly for cross pollination leading to prevalence of appreciable genetic diversity that needs to be consolidated and characterized for profitable utilization. Further studies are however necessary to establish mating systems and if geitonogamy is totally excluded resulting in true out-crossing, permitting intense genetic introgression in natural stands of this species.

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