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Diversity, distribution and conservation of Saffron Thistle (*Carthamus lanatus* L.) in mid-high altitude temperate zone of Jammu and Kashmir, India: A DIVA-GIS study

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Abstract: The paper describes the diversity and distribution of wild safflower (*Carthamus lanatus* L.) from parts of Kashmir valley in India. Two exploration missions were carried out in four districts *viz*., Anantnag, Baramulla, Budgam and Pulwama and thirty-two accessions of saffron thistle germplasm were collected and conserved. Diversity was observed in plant height (102.4 - 142.6 cm), capitula per plant (8 - 59), seed length (2.8 - 4.2 mm), seed yield per plant (5.4 - 21.8 g) and seed test weight (2.5 - 3.6 g). The highest coefficient of variation was found in number of capitula per plant followed by seed yield per plant, plant height, seed width, 100-seed weight and seed length. DIVA-GIS grid maps were generated for the diversity analysis of select traits and described. Highest Shannon diversity indices (2.0-3.0) for plant height, number of capitula per plant and 100-seed weight were recorded for the saffron thistle germplasm accessions collected from some areas of Pulwama and Budgam districts of Kashmir valley. South-western region of Kashmir valley has thus been identified as diversity-rich pockets for saffron thistle in India. The collected germplasm of saffron thistle was deposited in the National Gene Bank located at NBPGR, New Delhi and conserved under long-term conditions for its utilization in crop improvement programmes in the country.

Resumen: El artículo describe la diversidad y la distribución delcardo lanudo (*Carthamus lanatus* L.) en partes del valle de Cachemira en la India. Se llevaron a cabo dos misiones de exploración en cuatro distritos (Anantnag, Baramulla, Budgam y Pulwama) y se recolectaron y conservaron 32 adquisiciones de germoplasma de esta especie. La diversidad se evaluó a través de la altura de la planta (102.4 a 142.6 cm), el número de capítulos por planta (8 a 59), la longitud de las semillas (2.8 a 4.2 mm), el rendimiento de semilla por planta (5.4 a 21.8 g) y el peso de prueba de las semillas (2.5 a 3.6 g). El coeficiente de variación más alto se encontró en el número de capítulos por planta, seguido del rendimiento de semillas por planta, la altura de la planta, el ancho de semilla, el peso de 100 semillas y la longitud de la semilla. Se produjeron y describieron mapas de retícula con el programa DIVA-GIS para el análisis de la diversidad de los rasgos seleccionados. Se registraron los mayores índices de diversidad de Shannon (2.0-3.0) para la altura de la planta, el número de capítulos por planta y el peso de 100 semillas para las adquisiciones de germoplasma de cardo lanudo procedentesde áreas de los distritos Pulwama y Budgam del valle de Cachemira. Por lo tanto, la región sur occidental del valle de Cachemira fue identificada como un reservorio rico en diversidad de cardo lanudo en la India. El germoplasma recolectado de cardo lanudo fue depositado en el Banco Nacional de Genes situado

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en NBPGR, Nueva Delhi, y conservado en condiciones de largo plazo para su utilización en programas de mejoramiento de los cultivos en el país.

Resumo: O artigo descreve a diversidade e distribuição de cártamo-bastardo (*Carthamus lanatus* L.) em partes do vale da Caxemira, na Índia. Foram realizadas duas missões de exploração em quatro distritos viz., Anantnag, Baramulla, Budgam e Pulwama e trinta e dois acessos de germoplasma de cártamo foram coletados e conservadas. A diversidade foi observada quanto à altura da planta (102,4 - 142,6 cm), capítulos florais por planta (8 - 59), comprimento da semente (2,8 - 4,2 mm), o rendimento de sementes por planta (5,4 - 21,8 g) e peso de sementes de teste (2,5 - 3,6 g). O maior coeficiente de variação foi encontrado no número de capítulos florais por planta seguido do da produção de sementes por planta, altura da planta, largura da semente, peso de 100 sementes e comprimento da semente. Com o DIVA-GIS foram gerados mapas com grelhas para a análise da diversidade dos caracteres selecionados e descritos. Os maiores índices de diversidade de Shannon (2,0 - 3,0) para a altura da planta, o número de capítulos florais por planta e peso de 100 sementes para osacessos de germoplasma de cártamo-bastardo coletados de algumas áreas dos distritos de Pulwama e Budgam do vale de Caxemiraforam registados. A região sudoeste do vale de Caxemira foi assim identificada com bolsas ricas em diversidade de cártamo-bastardo na Índia. O germoplasma de cártamo-bastardocoletado foi depositado no Banco Nacional de Genes localizado no NBPGR, Nova Deli e conservado sob condições de longo prazo para a sua utilização em programas de melhoramento de culturas no país.

Key words: Asteraceae, *Carthamus*, crop improvement, crop wild relative, DIVA-GIS, germplasm, Kashmir.

Introduction

Wild species especially wild relatives of crop plants are invaluable source of resistance to several biotic and abiotic stresses, yield, nutritional quality, adaptation and genetic diversity. However, their utilization in crop improvement programmes depends largely on their availability as well as their crossability relationship with the cultivated types (Pandey *et al.* 2005). India is endowed with thousands of economically useful wild plant species. Wild relatives of crop plants belonging to the genera such as *Avena, Carthamus, Cicer, Juglans, Olea, Trigonella, Vigna* etc. are distributed in Kashmir (Arora & Nayar 1984). Saffron thistle (*Carthamus lanatus* L.) is one such closely related wild and weedy species of cultivated oilseed crop safflower (*Carthamus tinctorius* L.) which is quite widely distributed in the Kashmir valley. It is a native species of Mediterranean region, southern Europe and Central & West Asia and has spread to many temperate regions of the world including USA, Argentina, Chile, New Zealand, Australia, Greece, Morocco, Portugal, South Africa, Turkey, Uruguay and some other countries including temperate regions of India (Weiss 1971).

Kashmir valley is the central part of North Western Indian Himalayan state of Jammu & Kashmir extending between 32° 17′ N to 37° 05′ N and 72° 31′ E to 80° 20′ E. Topographically Kashmir with a moderate climate is deep elliptical bowl-shaped valley bounded by lofty mountains of Pir Panjal in the south and southwest and the Great Himalayan range in the north and east with 64 % of total area being mountainous. It has an average annual precipitation of 660 mm with average temperature around 13°C and the altitude ranging between 1500 - 2500 m asl. The physiography of target districts is highly unequal. The high hills and the elevated plateau of alluvial and lacustrine materials are present in the study area. These are locally known as *Karewas* (Wudars). The *karewas* are divided from each other, sometimes cut into strips by valleys from 30 - 92 m (100 to 300 feet) deep, occasionally they are surrounded altogether by lower ground but more generally they connect on to some of the mountains that bound the ravine. The *karewas* of study area are famous for the cultivation of saffron, apples and

almonds. Besides, major crops grown in these districts are rice, wheat, maize, vegetables and fruits and oil seeds (Bhat 2013).

Carthamus lanatus L. (Family: Asteraceae) also known as *woolly distaff thistle, false star thistle, woolly safflower* and *woolly star thistle* is a problematic highly prickly plant commonly occurring on dry *karewa* agricultural lands throughout Kashmir, locally called "*Tchari kund*". The seeds germinate in May producing a rosette with spiny leaves. The flowering stems develop in June, flowering occurs in July and seeds mature in August. The stems of young plants are usually covered with loose woolly or cobwebby hairs. The plant is erect, often much branched annual growing up to 1 - 1.5 m tall. Stems usually single at the base, branching apically, sparsely septate-hairy, cobwebby, glandular and striate. Leaves alternate, sessile, triangular and deeply pinnatified with a prominent strong spine at tips. Rosette leaves are narrow about 20 cm long clasping the stem. Stem leaves shorter, rigid, stem clasping and armed with stout spines. Flower heads with sulfur - yellow florets are surrounded by spiny bracts, borne at the ends of the branches. Seeds are brownish-grey, 3 mm long with a four-angled base, sometimes with a pappus of stiff bristles. Each capitulum produces 10 - 16 seeds. Saffron thistle has an unbranched tap root system. Seed buried in the soil may survive for over eight to ten years but hardly ever geminates at depths below 5 cm. Most seeds germinate within 3 years of release but the seed has complex dormancy characteristics (Kessler 1987).

Conservation biologists and researchers paid less attention to this wild and problematic plant species until recently. Destruction of habitat and changing land use pattern has led to its genetic erosion. In the present global scenario, changing climate regimes, unsustainability of high input agriculture, search for novel genes for food and nutrition, biotic and abiotic stresses have increased the focus on augmentation, conservation and utilization of such wild taxa. Hence, an attempt has been made to study its distribution and diversity in temperate zone using Geographic Information System (DIVA-GIS), augmentation of germplasm and conservation aspects.

Methods

In order to capture the endemic genetic diversity in wild safflower *Carthamus lanatus*, two exploration and collection missions were organized during August/September 2012 & 2013 in parts of Kashmir valley. Out of the 10 administrative districts of Kashmir, 4 districts Anantnag, Baramulla, Budgam and Pulwama were covered in the present study. The planning and logistics and the sampling procedures for the collection of wild safflower germplasm follows Engels *et al*. (1995) and Brown & Marshall (1995). Since *Carthamus lanatus* occurs in wild state only, random samples of the populations and biased samples of elite material with comparatively large sized seeds were collected. Germplasm of woolly safflower was collected mostly from *Karewas* and pasture lands in its wild status. Geographical coordinates of the collection sites were recorded using handheld global positioning system (Garmin 12). Ethno-botanical information on woolly safflower was also collected from old folks/shepherds. Passport data for the collected germplasm were recorded and documented. Standard descriptors for wild safflower (IBPGR 1983) were used for studying the variability in the collected germplasm. SAS enterprise guide version 4.3 was used for statistical analysis. DIVA-GIS version 7.5.0 was used for mapping diversity and analysis of saffron thistle.

Results

Saffron thistle, a wild relative species of safflower, *Carthamus tinctorius* is widely distributed in the Kashmir valley. The two germplasm collection missions during 2012 and 2013 resulted in augmenting 32 accessions of woolly safflower from the mid-high altitude temperate zone of Jammu and Kashmir covering Anantnag (2), Baramulla (2), Budgam (13) and Pulwama (15) districts (Table 1). The collected germplasm of saffron thistle was conserved in the National Gene Bank located at NBPGR, New Delhi, under long term conditions (-20 °C, RH 15 % and seed moisture content reduced to 4 - 6 %). A set of germplasm accessions are also being maintained at medium term modules at Akola and Hyderabad and at ambient temperature conditions at Srinagar regional station of NBPGR. The actual collection sites of saffron thistle germplasm in Kashmir valley are presented in Table 1. *Carthamus lanatus* can be commonly seen growing on *karewas* (elevated table-land of lacustrine soils of lake-laid clays and shales to the south and west of

Table 1. Wild safflower (*Carthamus lanatus* L.) germplasm collected from Jammu & Kashmir.

Collection sites/Villages	District	No. of accessions collected	Accession identity
Shangus, Mattan	Anantnag	2	IC597261, IC597262
Kichhama, Gantmulla	Baramulla	2	IC597265, IC597266
KD Farm, Kakaring			IC597249, IC597250,
Chrarisharief, Tujan,			IC597251, IC597252,
Bunhar, Dhoor Khansaheb,			IC597263, IC597264,
Old Airfield, Bunhar Karewa	Budgam	13	SHEIKH -23 to 29
Kand daji Hari Awantipur,			IC597245, IC597246,
Hapatdankad Hari Awantipur,			IC597247, IC597248,
Koel, Goose Karewa,Wuyan			IC597253, IC597254,
Pampore, Chewa Kalan,			IC597255, IC597256,
Lethpora, Boh Awantipur,			IC597257, IC597258,
Ladhu Pampore,			IC597259, IC597260,
Tral Chek, Khrew Pampore	Pulwama	15	SHEIKH -30 to 32

Fig. 1. A-D. A. Wild habitat showing the distribution of *Carthamus lanatus* L. in weedy form in Kashmir valley. B. Rosette leaves on young plant of *C. lanatus*. C. *C. lanatus* with more number of capitula per plant. D. Mature capitulum showing seeds with pappus hairs ready for dispersal.

Table 2. Descriptive statistical analysis on select traits of *Carthamus lanatus* L.

Trait	Minimum	Maximum	Mean ± SE	SD	CV %
Plant height (cm)	102.4	142.6	121.9 ± 4.9	15.6	12.8
No. of capitula plant^{-1}	8.0	59.0	22.6 ± 4.8	15.3	67.8
Seed length (mm)	2.8	4.2	3.7 ± 0.1	0.4	10.5
Seed width (mm)	2.2	3.2	2.8 ± 0.1	0.4	12.7
Pappus length (cm)	1.0	1.2	1.1 ± 0.0	0.0	3.4
Seed yield plant^{-1} (g)	5.4	21.8	13.1 ± 1.8	5.7	43.8
100-seed weight (g)	2.5	3.8	3.0 ± 0.1	0.4	12.5

the valley). It is very hardy, very rigid and prickly plant, and has less value as a fodder species. Younger plants are grazed by cattle and sheep but once the plant matures it is avoided by them. Various growth stages of saffron thistle as distributed in Kashmir valley are depicted in Fig. 1.

Variability has been observed for various traits viz., plant height, capitula per plant, seed length, seed yield per plant and seed test weight. The descriptive statistical analysis for various quantitative traits is provided in Table 2. The highest coefficient of variation was found in number of capitula per plant followed by seed yield per plant, plant height, seed width, 100-seed weight and seed length. DIVA-GIS grid maps were generated for diversity analyses of a few quantitative traits in saffron thistle viz., plant height (Fig. 2 A), number of capitula per plant (Fig. 2 B), 100-seed weight (Fig. 2 C) and pappus length. Highest Shannon diversity indices (2.0 - 3.0) for plant height, number of capitula per plant and 100 - seed weight were recorded for the saffron thistle germplasm accessions collected from some areas of Pulwama and Budgam districts of Kashmir valley. The results indicate that diverse accessions for all these traits excepting pappus length can be sourced from south-western region of Kashmir province of Jammu & Kashmir state of India and these regions are diversity-rich pockets for saffron thistle in India. Grid map generated for diversity analysis in pappus length indicated that no significant diversity exists for the trait (Table 2).

Discussion and conclusion

The wild ancestors of crop plants possessing genes having great potential for their utilization in crop improvement programmes are increasingly being used as sources of genetic diversity to breed new crop varieties suitable for changing climates; requiring less fertilizer and few energy inputs and resistant to new pests and diseases (Maxted *et al*. 1997). In a review of the crop wild relatives in 29 major crop species, 183 wild taxa have been used in breeding (Maxted & Kell 2009) and the future plant breeding will require such broadening of the genetic base if we are to meet the challenges of 21st century (Feldmen & Sears 1981; Gepts 2004). The most widespread use of crop wild relatives has been the development of disease and pest resistance, with 39 % of use associated with improving disease resistance, 17 % with pest resistance, 13 % with abiotic stress, 10 % with yield increase, 11 % with quality improvement, 6 % with husbandry improvement and 4 % with cytoplasmic male sterility and fertility restoration (Maxted & Kell 2009). *Carthamus lanatus*, the crop wild relative of cultivated safflower is an obnoxious prickly plant competing with other plant taxa for resources and input such as moisture, light and nutrients. It is estimated that in other countries, infestation of woolly safflower in cereals and millets would cause yield reductions up to 70 %. It is regarded as a pest in Australia, USA, Europe and other countries. However, this species will be of little threat in the Kashmir valley as of now. Being viewed only as a problematic weed, saffron thistle has not been exploited for oil and yellow dye in this region. It has 16 - 20 % seed oil content with Iodine value of 138 - 144 (Weiss 1983). During our survey, few old folks and shepherds have revealed that the dried plants of *Carthamus lanatus* were burnt and the ashes used for curing wounds. However, this traditional practice needs to be validated for active principles. Infact, *Carthamus lanatus* has been reported to have antibacterial and antifungal activity (Taskova *et al*. 2002). This wild relative of cultivated safflower is an invaluable source of resistance to *Alternaria* leaf spot, bacterial blight, *Fusarium* wilt and rust diseases (Heaton & Klisewicz 1981; Zimmer 1967). It has also been reported to carry genes for resistance against safflower fly (Kumar 1993). Hence, genome resources of saffron thistle may be effectively utilized in

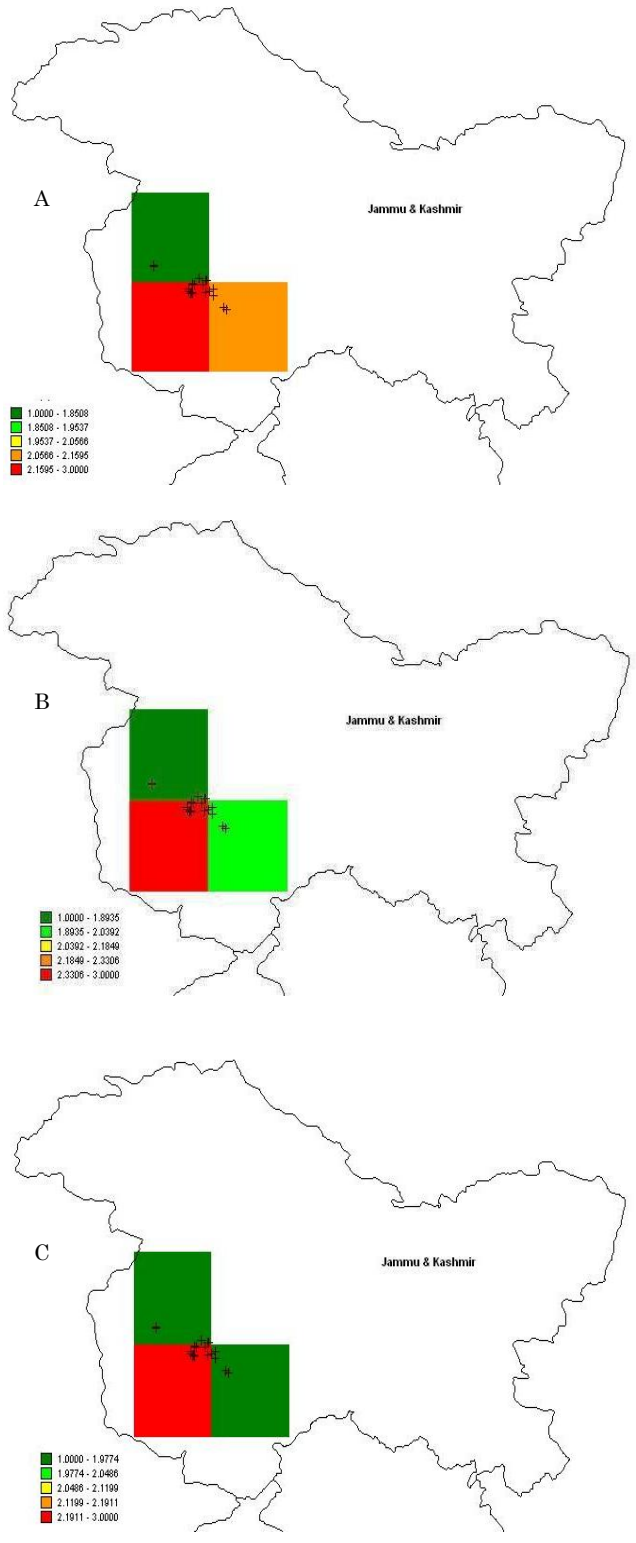

Fig. 2. A-C. DIVA-GIS grid maps for diversity in plant height (A), capitula per plant (B) and test weight (C) in *Carthamus lanatus* from mid-high altitude temperate zone of Jammu & Kashmir.

safflower crop improvement programmes in the country.

Saffron thistle in Kashmir has tremendous variability for traits - number of capitula, seed yield and plant height as revealed in the present study. It is considered to be cold and drought resistant (Duhoon 1999). Indigenous accession of *Carthamus lanatus* collected from Kashmir valley, India has been reported to possess higher content of oleic acid (23.2 %) and palmitic acid (9.7 %) (Murthy & Anjani 2008). Effective and methodical exploitation of these traits is the key to safflower crop improvement program. Interestingly, high coefficient of variation (67.8 %) was obtained for trait, number of capitula per plant in our study. Similar variability was recorded earlier in cultivated safflower by many researchers (Dikshit *et al.* 2012; Ghorpade *et al.* 1993; Pahlavani 2005; Pandravada *et al.* 2012).

The recent advancement in the fields of Remote Sensing & Geographic Information System, mobile computing, GPS technology, wireless connectivity, and internet has enabled accurate and uniform documentation of biological diversity with revisit capability helping in identification of the hotspots of biodiversity and the gaps in biodiversity exploration (Roy *et al.* 2012). DIVA-GIS, a Geographic Information System (GIS) used for mapping diversity and analysis of saffron thistle in the present study was designed to map the range of distribution of species (Hijmans *et al.* 2000). It enables us to understand and comprehend the distribution of diversity including wild species on the geographical scale and also helps in identifying gaps in collection. GIS tool can be effectively used for diversity analysis, assessment of loss of diversity, developing new strategies for conservation and sustainable utilization of wild species. DIVA-GIS tool has been successfully used in identifying areas of high diversity in *Phaseolus* bean (Jones *et al.* 1997); wild potatoes (Hijmans & David 2001); horsegram (Sunil *et al.* 2008); *Jatropha curcas* (Sunil *et al.* 2009); sesame (Spandana *et al.* 2012); linseed (Sivaraj *et al.* 2009); blackgram (Babu Abraham *et al.* 2010); *Canavalia* fatty acids (Sivaraj *et al.* 2010) and agrobiodiversity (Varaprasad *et al.* 2008). In a more recent study bioclimatic indices have been generated from monthly temperature and precipitation data using BIOCLIM model implemented in DIVA-GIS (ver. 7.1.7.2) for modelling environmental niche of Himalayan birch (Singh *et al.* 2013). Grid maps generated for diversity analysis in the present study indicate that south-

western regions including parts of Pulwama and Budgam districts of Kashmir province of Jammu and Kashmir are diversity-rich pockets for saffron thistle in India. Congenial habitat of dry *Karewa* lands is prevalent in these areas. During the course of our study 32 germplasm accessions of saffron thistle have been collected mostly from these areas. The collected germplasm was conserved in the National Gene Bank located at NBPGR, New Delhi, under long term conditions. A set of germplasm accessions is also maintained at medium-term modules at Akola and Hyderabad and at ambient temperature in Srinagar regional stations of NBPGR. Few previous germplasm accessions of *Carthamus lanatus* were collected by Duhoon (1999) from Chandhara (Pulwama) and Singhpur (Baramulla) in Jammu and Kashmir. One accession (IC-339691) was collected from Pulwama district in 2001 during World Bank funded subproject on Sustainable Management of Plant Biodiversity under National Agricultural Technology Project (1999-2005). Unfortunately, during last few years due to habitat loss the populations of *Carthamus lanatus* has shown a declining trend, more and more of these lands are now brought under cultivation of different crops. Therefore, concerted efforts are to be made for *in-situ* conservation of wild *Carthamus* species through protection of habitats and ecosystems in Kashmir valley to ensure its possible role as a source of desirable genes in future.

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