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Khesari (*Lathyrus sativus* L.), an ancient legume for future gain: An expedition collection from parts of West Bengal state of Eastern India

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Grasspea is one of the staple foods of the local people living in the eastern parts of India. An expedition was undertaken during March, 2020 to collect germplasm of grasspea in the lower-Gangetic riverine belt and coastal areas of West Bengal of eastern India lying between latitude 21.43-24.44°N and longitude 87.23-88.90°E. From the results of a structured questionnaire administered to grasspea farmers in 57 villages located in 96 local government areas, it appeared that grasspea is the primary winter pulse cultivated in this region. Large variability of germplasm exist, ranging from small to bold seed, early to late maturing types, moderate to the high biomass type of grasspea. Most of these landraces have been adopted over the years from neighbouring communities, but in a few instances, the varietal replacement was noted, which came either through the involvement of government departments or local seed dealers. The highest proportion of the accessions (52.38%) was collected from the Purba Medinipur district, and the lowest (19.05%) was from Paschim Medinipur. On-spot evaluation of morphological traits, variations was detected in the descriptor characteristics across the locations. A total of 21 accessions was collected and assessed on-spot for different characters, viz., the seed's size, shape, seed colour, taste and texture revealed significant variation. The implications of this survey results for grasspea improvement in India are discussed in the present study.

Keywords: Climate resilient, Collection, Exploration, Grasspea, Khesari, Landraces

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Climate change mitigation requires resilient crops. In order to get maximum advantages, legumes should be more productive with a minimum input responsive and perform in an array of soils and climates. The grasspea, *Lathyrus sativus* (L.), an orphan legume, has received attention over the past decade due to its suitability to adverse environments and a source of high levels of proteins[1,2]. Grasspea is a geographically versatile crop and adapted to a vast range in the tropics of South Asia and Africa. Where no other crop can grow in a fragile ecosystem, it gives a good return. Due to its climate resilience, this legume is considered as an "insurance crop" having endurance in many regions of the globe under marginal land rendering economic, social and nutritional security to the poor farmers. Grasspea is locally known as *Khesari*, *Teora, Kasari*, *Khesari Kalai*, *Kalai* etc. It has grown in the Indian subcontinent since time immemorial, as evidenced by the archaeological excavations at a Harappan site, *Kanmer*, presently in Gujarat[3]. It is a prevalent low input winter legume crop in south-east Asia for utilization of the rice fallow areas. Its acreage, however, reduced from 1.27 m ha (1980-81) to 0.38 m ha in 2017-18 due to restriction imposed by the Indian government as it has neuroexcitatory compound, β-N-oxalyl-l-α, β diaminopropionic acid (β-ODAP), which is believed to cause Lathyrism. The attack of lathyrism is sudden and resulted due to cold exposure. Despite its ban, *Khesari Kalai* is still grown mainly in the dry tract of Chhattisgarh, Bihar, West Bengal, Maharashtra and Odisha states.

However, it is now gaining favour due to multiple benefits viz., low cost of cultivation, fitness to conservation agriculture, low carbon and water footprint, good source of protein and homoarginine, adaptation to harsh environments and crucially, its

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dual-purpose use as food and livestock feed. West Bengal produced 1,08,300 tonnes of grasspea in an area of 87,000 ha with a productivity of 1245 kg/ha, which is more than the national average yield of 804 kg/ha[4]. In this state, grasspea is mainly grown in rice fallow areas as a "relay crop" or "paira crop" and the seed is broadcasted into a standing rice crop before harvesting to utilize the residual moisture for good germination and zero tillage in cultivation[5]. Due to these adaptive advantages of this crop and low input requirements, it thrives well in the rice fallow areas of eastern India[6]. Being a leguminous crop, it has a high biological nitrogen fixation rate and can fix 67 kg/ha of atmospheric nitrogen in a season, which assists in maintaining soil fertility[7].

Harnessing the potential of underutilized germplasm is a vital endeavour in this aspect for developing improved cultivars. Prior to 2020, the National Bureau of Plant Genetic Resources (NBPGR) of Indian Council of Agricultural Research (ICAR) had gathered eight germplasm accessions of *Lathyrus sativus* from West Bengal. However, small and fragmented land holding limits the extensive utilization of this germplasm in comprehensive research programmes. To expand the historical Indian collection and site-specific collection, the NBPGR conducted a crop and site-specific exploration programme to collect grasspea from West Bengal to incorporate into the national genebank, adopting the principles and guidelines of the International code of conduct for plant germplasm collection and transfer of material. This grasspea germplasm collection mission were undertaken to capture the novel genetic variability for developing eco-friendly, productive varieties fitting in Indian condition. Another objective was to document indigenous traditional knowledge from the farmer folks of West Bengal who have been engaged in cultivating grasspea since long back. The justification of choosing West Bengal for germplasm exploration was its geographic position of sharing a border with Bangladesh, where grasspea is the most massive growing pulse.

Methodology

Planning

Passport data of grasspea germplasm have been analyzed to know the diversity affluent areas, trait-specific, potential areas, and identify collected vs conservation gaps. A gap analysis was also carried out to select districts and areas not represented in the Indian National Genebank collections, ICAR-NBPGR, New Delhi. A preparatory study reviewed herbarium specimens from the Botanical Survey of India, Kolkata (BSI) and ICAR-NBPGR, New Delhi. To locate probable sites, satellite images and green fields available through Google maps were studied. So far, 2,528 accessions of grasspea have been gathered from diverse agro-ecological zones of India through crop-specific and multi-crop explorations (http://genebank.nbpgr.ernet.in/CropSpecieswithICECWise.aspx?CropCode=1641). These explorations were conducted independently or collaboratively with crop-based Institutes/ State Agricultural Universities/ Krishi Vigyan Kendras. Geo-referenced maps were prepared with WGS84 datum and geographic projection systems using GIS tools[8] (Fig. 1). Relevant authorities, such as the Project Coordinator, ICAR-All India Coordinated Research Project on, (MULLaRP) ICAR-Indian Institute of Pulses Research, Kanpur, State Department of Agriculture, the Government of West Bengal and the International Center for Agricultural Research in the Dry Areas (ICARDA) the project coordinator, ICAR-All India Coordinated Research Project on, (MULLaRP) ICAR-Indian Institute of Pulses Research, Kanpur, also consulted about the status and prospects of grasspea in West Bengal. To capture maximum diversity, planning activities like survey, route and liaisoning with local workers were done to explore the targeted areas before starting the collection trip.

To collect grasspea germplasm, this exploration mission was undertaken by ICAR-NBPGR in collaboration with crop breeders from Bidhan Chandra Krishi Viswavidyalaya (BCKV), Nadia, to explore grasspea cultivation areas in Paschim Medinipur, Purba Medinipur and Murshidabad districts of West Bengal during March, 2020. Grasspea landraces were collected from 21 sites between 21.43-24.44 N latitude and 87.23-88.90 E longitude. Flood prone areas of rivers- Ganga, Rupnarayan and the coastal areas adjacent to the Bay of Bengal were identified for exploration. A total of 17 blocks were surveyed during the collection mission. Appropriate sampling procedures were adopted to capture maximum allelic variations from different habitats/vegetation[9]. Fully matured pods of 50-60 plants were harvested following random sampling and bulked thereafter. During sampling, care was taken to collect samples from all morphotypes observed in the field. Collected materials and passport data were submitted to NBPGR, New Delhi (Supplementary Material-1).

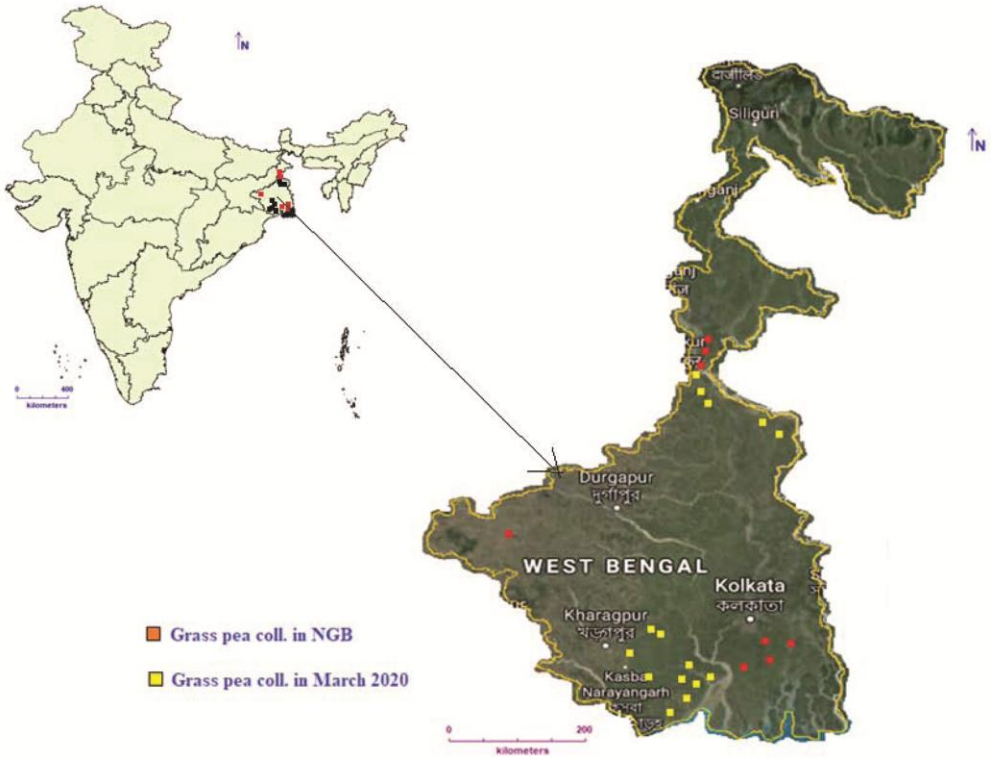

Fig. 1 — Grasspea germplasm collection site

On-site data recording

A strategic protocol was developed to record on-site data using grasspea descriptors during the trip. Fresh seeds from matured pods were collected only from those plants with a different type. At each collection site, selected phenotypic traits and associated climatic factors were noted besides GPS co-ordinates. The important agronomic traits recorded included accession type, flower colour, seed weight (g), pattern, seed/ pods, days to maturity, plant height (cm), biomass and disease, and pest incidence. Stepwise workflow for the germplasm collection is depicted in Figure 2.

Agro-morphological traits were recorded at the time of collection of germplasm using grasspea descriptors[10]. A total of nine qualitative and five quantitative traits were recorded. The presence of biotic stresses was also noted. Standard passport data (Supplementary Material-2) (ICAR-NBPGR) of each accession in consultation with farmers and a note on specific uses and traditional knowledge, was also recorded at collection sites**.**

Results and Discussion

Germplasm collection

In India, the first and foremost systematic effort to collect the grasspea landraces was initiated in 1967.

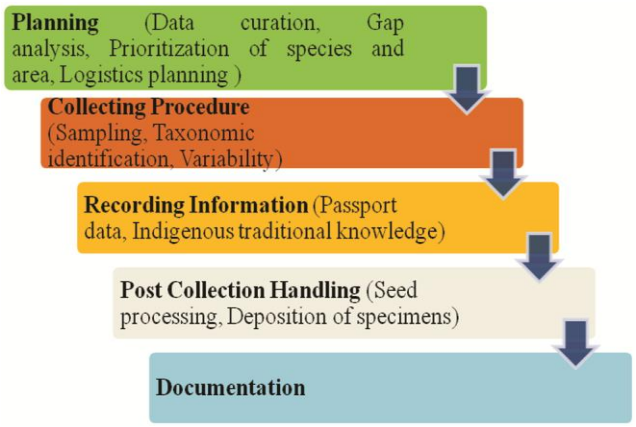

Fig. 2 — Schematic workflow depicting the steps followed in collecting grasspea germplasm

Subsequently, collections were made from grasspea growing regions of India, including tribal areas of erstwhile Bihar. In 1976, selected native germplasm was used in the Grasspea Improvement Program initiated at Raipur, Chhattisgarh. Later, the Directorate of Pulses Research, Kanpur, supplied some indigenous materials to enrich the programme. Exotic accessions from grasspea dominating countries were also introduced by NBPGR, New Delhi. During 1990 and 1991, many grasspea landraces were collected from the unexplored areas of Central India.

Grasspea germplasm has been collected subsequently in sporadically and multi-crop explorations in different parts of India. Currently, 2,619 accessions are maintained at the Indian national genebank, ICAR-NBPGR. During 1994-95, 283 accessions of different *Lathyrus* species were assessed for agronomic traits at ICAR-IARI, New Delhi[11]. In the present exploration, 21 accessions of *Lathyrus sativus* were collected from three districts covering Paschim Medinipur (4), Purba Medinipur (11) and Murshidabad (6) belonging to the rice fallow areas of coastal saline zone, eastern Gangetic old alluvial and new alluvial zone, respectively. To withstand drought, salinity and moisture stress, grasspea has developed a deep penetrating root system and modifications like narrow leaves and winged stem for a reduction in transpirational losses. These specific habitat niches of coastal areas highlight the potential of grasspea in alleviating salt stress and thus highlight it as a vital donor in breeding for abiotic stress tolerance. Grasspea with good early biomass and dominating nature has a unique capacity to overcome weed infestation. Though, during germplasm collection, species such as Bengal vetch (Hetka) *Vicia benghalensis* (L.) was identified as a common weed in field boundaries, fallows and roadsides while intercropped with rapeseed-mustard, linseed, lentil, chickpea and oats (3:3 or 3:2 cropping ratio).

In Figure 1, the geo-referenced map showed the germplasm collecting sites of grasspea. Both past and present germplasm collection sites are depicted on the map. Past accessions conserved in National Genebank have shown in red colour points while yellow colour points reflect the newly collected samples of grasspea from different micro-climatic regions of West Bengal. These accessions varied in seed shape, size, colour and uses. Indigenous knowledge on named grasspea landraces were also gathered through interaction with the local farming communities. The germplasm material collected has been deposited to the concerned division for processing and conservation under MTS/LTS and seeds specimen also deposited at National Herbarium of Cultivated Plants at NBPGR.

On-site data recording

On-site phenotyping of 21 landraces of grasspea was performed during an exploration trip. A total of 14 morphological traits were recorded for exhibiting variability among the collected accessions. IPGRI descriptors was used to characterize collected landraces. During morphological characterization, significant variability was observed in fields while recording data on attributes such as plant height, seed weight, seeds per pod and days to maturity (Table 1). Hundred seed weight ranged from 4.1 g to 6.54 g and the highest seed weight was recorded in the landrace belonging to Murshidabad (IC0634672). Accessions collected from Paschim and Purba Medinipur matured earlier than those collected from Murshidabad. Landraces of Medinipur were small-seeded, short in plant height, low to medium biomass whereas, landraces collected from Murshidabad had high biomass and number of seeds per pod. Murshidabad is one of the most fertile riverine tracts of West Bengal and is considered as the "crop museum" due to its favourable climate and edaphic factors suited to multiple crops[12]. Soils of Murshidabad are mainly resultant depositional action of the Ganges and its tributaries, which are highly fertile thus, landraces collected from this district had good biomass[13,14,15].

In qualitative traits, no distinct variation was observed among the accessions with respect to cotyledon colour; after removing seedcoat, all the accessions revealed yellow cotyledons. Seeds of majority of accessions were rhomboid-shaped while only two accessions (IC0634655 and IC0634673) had obtriangular seed shape (Fig. 3). In Indian grasspea collections, the predominating seed shape is rhomboid[16]. However, varying seed shape of grasspea like globose, subprolate type seeds with glossy, reticulate–foveate surface shapes had reported[17].

A tubercular seed surface was observed in 17 landraces. The majority of accessions had grey mottled seed coat surface, dotted seedcoat pattern, brown seedcoat pattern with the pigmented pod. Earlier studies reported that black, grey, grey mottled, grey white and yellow white coloured seeds were most frequently used among the Indian accessions[18]. Identifying agronomically superior and prominent qualitative traits is a prerequisite for successfully utilising grasspea landraces in crop improvement[19]. Morphological variability in the seed is depicted in Figure 4.

Table 1 — Descriptive statistics for quantitative traits

S. N.	Traits	Minimum	Maximum	Mean±SE	CV (%)
1	100 seed weight (g)	4.1	6.54	5.46 ± 0.12	10.03
2	Seed diameter (cm)	0.31	0.51	0.43 ± 0.01	13.71
3	Plant height (cm)	81.66	133	96.31 ± 3.88	18.45
4	Seeds/ pod	3	6	3.95 ± 0.21	24.64
5	Days to maturity	98	123	105.52 ± 1.88	8.16

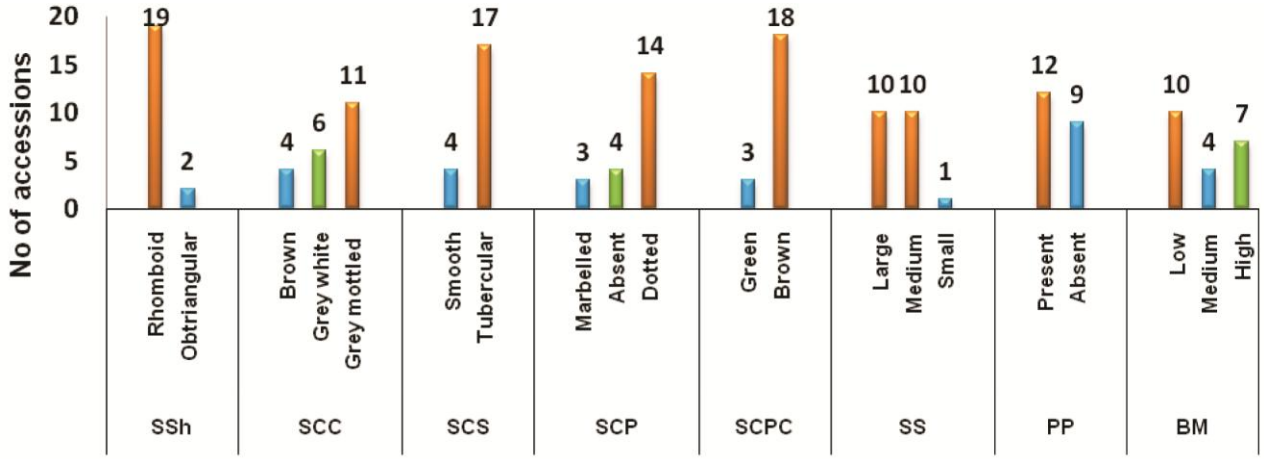

Fig. 3 — Variation in qualitative traits (*SSh Seed shape; SCC Seed coat colour; SCS Seed coat surface; SCP Seed coat pattern; SCPC Seed coat pattern colour; SS Seed size; PP Pod pigmentation; BM Biomass*)

Fig. 4 — Variability in seeds of collected accessions of grasspea

The most significant advantages of grasspea cultivation are its least vulnerability to pests and diseases[20]. However, during the exploration mission, the occurrence of biotic stresses was also noted. Visual examination revealed the presence of three

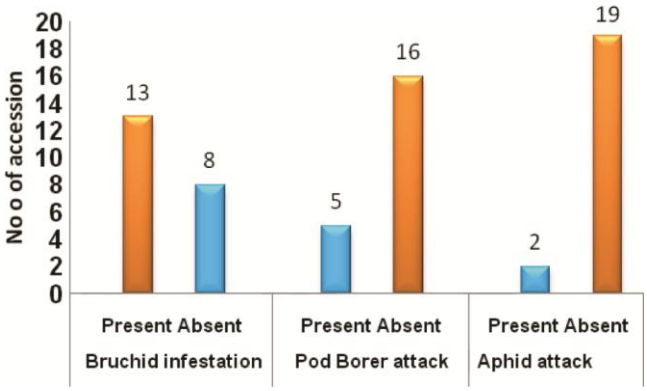

Fig. 5 — Occurrence of different insects on collected accessions

major pests in parts of the covered area *viz* Bean aphid (*Aphis craccivora*), Pod borer **(***Etiella zinckenella*) and Bruchids (*Callosobruchus chinensis*). Bruchids are major storage pest in pulses[21]. The farmers said that pod borer is emerging as a major threat to grasspea in the Medinipur region while bruchid is a major storage pest in grasspea. The presence of insect infestation on collected accessions is represented in Figure 5.

Utilization and traditional knowledge

Orphan legume like grasspea is primarily grown by resource-poor vulnerable section of the society to get nutritional sustenance to their food plate[22]. Grasspea is geographically successful legume and survival food for the poor. Unfortunately, as per IPGRI descriptor, observation of lathyrism on human being and livestock was added in the questionnaire and grasspea suffers from a reputation of toxic pulse[1]. However, there was no known report of lathyrism in covered districts despite consumption of grasspea since long

back. In other parts of India viz., Gondia district of Maharashtra state, the cases of neurolathyrism reduced drastically in all selected villages due to lesser β-ODAP exposure through *Lathyrus sativus* consumption[23]. In our survey, all the grasspea growers and the majority of the local people, who were interacted, reported consumption of grasspea in their regular diet in different forms without any adverse effect. Indian Council of Medical Research (ICMR) has given directives to use *khesari* is safe in a well-balanced diet with more than one-third of cereals to maintain the right amino acid[24]. Grasspea is also decorated with the term 3G, God Grace Grasspea (Dr Ashutosh Sarker, ICARDA; pers. com.).

Human consumption

Grasspea is mainly consumed as pulses in India. It has around 18–34% and 17% of protein content in seeds and mature leaves respectively[25]. Most of the fatty acids in grasspea are polyunsaturated; thus, highly suitable for human consumption[26]. Grasspea is tagged as functional food as it is only known dietary source of homoarginine[27,28]. It is rich in anti-oxidants. The dal lends crispiness to fried stuff (Fig. 6a). Preferences of different seedcoat colour depend upon tradition and use in different regions[29]. The whole grain of local khesari fetch low market price (50–60 ₹/kg; 0.8–1 US$) as compared to that of the split pulse (60–75₹/kg; 1–1.2 US$).

The tender young shoots of the pre-flowering stage are plucked and sold as a green leafy vegetable (Fig. 6b). Off-season use of rolled and dried leaves as a vegetable was also documented[30]. The immature green seeds as well as the whole pods are consumed directly. Grasspea leaves are highly-priced vegetables (150-200 ₹/ kg; 2-3US$) popular among the region's locals. As grasspea is a common adulterant of gram flour[31], it is added for taste and makes the entire cost of gram flour cheaper. Besan (Khesari flour) is lighter yellow. It is sweeter and more refined than other pulses (Fig. 6d) (pers. com.).

Grasspea flour imparts crispiness and taste after blending with other pulses. Grasspea flour in many parts of Bangladesh is used to prepare roti (unleavened bread) for landless labourers. Roasted seeds of grasspea are also sold in the local market

Fig. 6 — a-Grasspea split-grain for use as dal; b-grasspea tender twigs being sold as leafy vegetables along with other leafy vegetables; c-grasspea as fodder; d-pakora made from grasspea flour; e-fried grains of grasspea as snacks being sold in the local market along with other fried grain legumes; f- badi/pellets made from grasspea grain also called khesari badi

Fig.7 — Harvesting and threshing of grasspea in rice fallow areas

(Fig. 6e). Roasting of seed does not fully parch seed. The grounded grains are used in a pancake-like preparation of *Badi* or *pakoda* (Fig 6f) (pers. com.). *Khesari badi* can be prepared in one day where as, from other pulses it is made ready in two days. Durability of *Khesari badi* is also more than other pulses (pers. com).

Forage and livestock feed

Green foliages are good livestock feed. Grasspea has excellent potential as a fodder or green forage crop (Fig. 6c). Fodder yields is estimated at around 7-10 t/ha in intercropping with maize in studies at Bangladesh Agricultural Research Institute, Joydebpur[32]. It was reported that the potential nutrient efficiency of different leguminous crops, including grasspea was found to be changed with different stages of maturity[33]. Grasspea in various supplements is given to lactating cattle as green fodder and husk increase milking ability (pers. com.)

Boon for conservation agriculture

Grasspea loves can be grown in heavy soils in low-lying areas where no other crops can grow easily. In parts of South East Asia, this is cultivated as a 'utera' crop after winter paddy as a traditional practice[34]. The seeds are broadcasted into a standing rice crop 2-3 weeks prior to harvest, and the grasspea crop utilizes the residual moisture and soil nutrients for growth. Grasspea has good promise in rice-fallows[35] and at least 0.5 m ha area out of 11.6 m ha fallows of South East Asia can be taken under *Lathyrus* to make the choice of second crop available for farmers and improve soil fertility and productivity.

Generally, farmers preferred small-seeded grasspea over bold seeded in rice fallow areas because of the low water requirement of local germplasm with small seed size during germination. This is the main reason for adopting landraces or local germplasm of grasspea. Farmers also opt for hydropriming by soaking the seeds in water or a mixture of cattle dung. The crop needs no-tilling and little care. Farmers are using traditional means for harvesting and threshing, which is highly remunerative (Fig. 7). Grasspea is still a profitable crop than *boro* or summer rice in West Bengal. Since grasspea is animal-loving forage in this region, farmers have developed the traditional skill to cover the field by date palm (*Phoenix dactylifera* L.) leaves after sowing to reduce animal trespassing. Date palm leaves is hidden in the crop canopy, but get pricked by the sharp edges of the leaves, disallowing straying animal entering into the field. To prevent infestation of bruchids, seeds are mixed with tobacco leaves, chilli, turmeric, and neem extract during storage.

Conclusion

Grasspea is tagged as an ancient crop for future needs. By virtue, grasspea must survive with the extremes of drought and waterlogging stresses, salinity and nutrient-poor soils. Information on the *ex-situ* holdings of grasspea landraces acquired through PGR Portal (http://pgrportal.nbpgr.ernet.in/(S(2isx1l455tu5qt45ngs2r145))/default.aspx) indicated that present collections from these three districts were unique and new to Indian genebank, NBPGR, New Delhi. Landraces from Murshidabad with six seeds per pod is unique germplasm to cater to the needs of the breeding program as a yield-enhancing factor. High biomass accessions with forage potential can meet the gap between livestock feed supply and demand. It is accepted that landraces from diverse and different habitats tend to have greater resilience to

various stresses than those with a narrow range of distribution. Under natural conditions, aphid (caused by *Aphis craccivora*) and pod borer (*Etiella zinckenella*) are commonly prevalent in grasspea crop[20]. On-site data of germplasm collected indicated that local germplasm was almost 10-14 days earlier in maturity than released varieties. Early maturing accessions perfectly fit in the rice fallow system where often sowing of succeeding pulses is delayed due to late harvesting of long duration rice. Grasspea growing areas of West Bengal fall under the fragile ecosystem. Erratic cyclone and indentation of salty water leads to failure of all crops and also causes a reduction in yield of grasspea landraces. Now, it is need of the hour to promote khesari dal cultivation on rice fallows. This will help to resolve the shortage of pulses whose shooting prices have put them beyond the reach of the very poor. Conservation of landraces assembled from these threatened areas may aid to check the genetic erosion of local germplasm with unique and valuable traits. Grasspea continued to be the survival food for all in the developing world, and limited consumption is safe and healthy. Systematic and focused work needs to be done to evaluate grasspea genetic resources sleeping in genebanks to produce locally adapted low toxin varieties.

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Conflict of Interest

Authors unanimously proclaim that they do not have a conflict of interest.

Authors' Contributions

Conceptualization: KT, AS; Exploration and Collection of germplasm accessions: KT, RS, AD, KJ: Supervision: AK; Collection of geo-referenced data: DS; Writing - original draft: KT, AD; Writing - review & editing: RS, SA, AS.

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