Plant Genetic Resources: Characterization and Utilization

cambridge.org/pgr

Morphological description based on DUS characters and molecular characterization of 'Sikkim Primitive' maize: an endangered unique genetic resource

Chandan Kapoor[1], Shweta Singh[2], R. K. Avasthe[3], Chandramani Raj[2], Matber Singh[4] and Hissay L. Lepcha[5]

[1]ICAR-Indian Agricultural Research Institute, Pusa, New Delhi, India; [2]ICAR-Indian Institute of Sugarcane Research, Lucknow, UP, India; [3]ICAR Research Complex for NEH Region, Sikkim Centre, Gangtok, India; [4]ICAR-Indian Institute of Soil and Water Conservation, Dehradun, Uttarakhand, India and [5]Horticulture Department, Government of Sikkim, Gangtok, India

Short Communication

Cite this article: Kapoor C, Singh S, Avasthe RK, Raj C, Singh M, Lepcha HL (2022). Morphological description based on DUS characters and molecular characterization of 'Sikkim Primitive' maize: an endangered unique genetic resource. *Plant Genetic Resources: Characterization and Utilization* **20**, 69–72. https://doi.org/10.1017/S1479262122000065

Received: 15 April 2021
Revised: 12 April 2022
Accepted: 12 April 2022
First published online: 10 May 2022

Key words:
DUS traits; farmers' rights; landrace; maize; on-farm conservation; prolific maize; Sikkim Primitive; SSR marker

Author for correspondence:
Chandan Kapoor,
E-mail: chandannaarm@gmail.com

Abstract

'Sikkim Primitive' maize locally known as 'murali makkai' in Sikkim is a unique genetic resource exhibiting prolificacy and excellent popping capacity. Status of SP has reached extinction level due to its very small population size and neglected conservation efforts *in situ*. In an initial effort to conserve and revive this landrace, characterization and documentation was carried out with 31 morphologically assayed traits recorded at different growth stages along with molecular characterization with simple-sequence repeat (SSR) markers. Plants exhibited prolificacy (5–6 cobs/plant) and excellent popping capacity along with other distinct traits. Plants were tall with thin stem, loose drooping tassel with anthocyanin coloration present at the base of glumes and in brace roots. Cobs were medium sized carrying small seeds with low test weight (87.90 g). A total of 22 SSR markers show amplification in murali makkai with markers bnlg1083, umc1353, umc1128, bnlg1017, bnlg2077, umc2298 and umc2373 amplified unique amplicons ranging from 100 to 800 bp. The characterized set of traits and molecular characterization for murali makkai will facilitate in utilization for genetic improvement and maintenance of genetic purity.

Introduction

Sikkim is one of the north-eastern states of India harbouring impressive maize diversity including some unique maize landraces like 'Sikkim Primitive' (SP). Maize genetic resources of Sikkim have been mentioned in various studies with special mention of 'Sikkim Primitives' (Karuppaiyan and Avasthe, 2006; Prasanna *et al*., 2009; Prasanna, 2010; Sharma *et al*., 2010; Singode and Prasanna, 2010). 'SP' maize locally known as 'murali makkai' is a prolific landrace (5–6 cobs/plant) with small grains having an excellent popping ability (95%) and distinct features due to its resemblance to wild corn (Dhawan, 1964). This landrace has a history of around 10,000 years and is a source of prolificacy, pest resistance and drought tolerance due to its survival under unfavourable conditions (Mani *et al*., 1987). The status of this landrace has reached near extinction level due to neglected conservation efforts *in situ* (on-farm conservation). This makes it imperative to initiate conservation efforts along with proper characterization and documentation. As per our study, no report on its characterization based on distinctness, uniformity and stability (DUS) traits is available as on date and also few studies have been carried out on molecular characterization of SPs (Sharma *et al*., 2010). Descriptions made earlier on SP (Dhawan, 1964; Singh, 1977) have several drawbacks as such studies have not been carried out in their area of adaptation i.e. northeast hill region where it shows full expression of distinct traits and the published studies are too old for utilizing them as reference. Data on morphological descriptors of germplasm are an essential prerequisite for crop improvement programmes to have knowledge on the distinct traits. Furthermore, germplasm characterization facilitates in registration of farmers' varieties and for long-term storage in National Seed Repository.

Experimental material

Seeds of murali makkai were collected from farmers' field at Gyathang village of lower Dzongu in the north district of Sikkim (known to be the home of SP) located at an altitude of 1728 m amsl (27°47′30″N and 88°49′68″E). The collected sample was tested in research trials conducted during the spring season of 2018 and 2019 at Organic Experimental Research Farm

Fig. 1. (a) Murali makkai with five cobs, (b) tassel attitude, (c) cobs, (d) unpopped kernels and (e) popped kernels.

Table 1. DUS and agro-morphological traits of murali makkai

Sl. no.	Character	Description
1	Leaf: angle between blade and stem (on upper leaf just above upper ear)	Wide (<45°)
2	Leaf: attitude of blade (on leaf just above upper ear)	Drooping
3	Stem: anthocyanin colouration of brace roots	Present
4	Tassel: time of anthesis	Late (>55 days)
5	Tassel: anthocyanin colouration at base of glume	Present
6	Tassel: anthocyanin colouration of glumes excluding base	Present
7	Tassel: anthocyanin colouration of anthers	Present
8	Tassel: density of spikelets	Dense
9	Tassel: angle between main axis and lateral branches	Wide (>45°)
10	Tassel: attitude of lateral branches	Strongly curved
11	Ear: time of silk emergence	Late (>58 days)
12	Ear: anthocyanin colouration of silks	Absent
13	Leaf: anthocyanin colouration of sheath	Absent
14	Tassel: length of main axis above lowest side branch	Short
15	Plant height (cm)	Medium (150–180 cm)
16	Plant: ear placement	Medium
17	Leaf: width of blade	Medium
18	Ear: length without husk	Medium (10–15 cm)
19	Ear: diameter without husk	Small (1.93 cm)
20	Ear shape	Conico-cylindrical
21	Ear: number of rows of grains	Medium (10–12)
22	Ear: type of grain	Flint
23	Ear: colour of top of grain	Yellow
24	Ear: anthocyanin colouration of glumes of cob	White

(*Continued*)

https://doi.org/10.1017/S1479262122000065 Published online by Cambridge University Press

Table 1. (*Continued.*)

Sl. no.	Character	Description
25	Kernel: row arrangement	Spiral
26	Kernel: poppiness	Present
27	Kernel: sweetness	Absent
28	Kernel: waxiness	Absent
29	Kernel: opaqueness	Absent
30	Kernel: shape	Round
31	Kernel: 1000 kernel weight	Very small (87.90 g)
Agro morphological traits		
1	Days to 50% tasseling	86.0
2	Days to 75% maturity	131.0
3	Plant height (cm)	223.0
4	No. of cobs/plant	5.67
5	Cob length (cm)	8.33
6	Cob diameter (cm)	1.93
7	No. of seeds per cob	144.0
8	1000 kernel weight	87.90
9	Seed yield (kg/ha)	670.0

of ICAR Research Complex for NEH Region, Sikkim Centre, Tadong, Sikkim located at an altitude of 1320 m amsl. The experimental plot consisted of 20 rows of 5 m length sown at a planting geometry of 60×20 cm^2. Data were recorded for 31 characters as per guidelines for the conduct of test for DUS on maize (http://www.plantauthority.gov.in/pdf/GMaize.pdf) at various crop stages. The crop was raised under recommended organic package of practices. For molecular characterization total genomic DNA from the samples was extracted following the modified cetyltrimethylammonium bromide procedure (Doyle and Doyle, 1990). Twenty two simple-sequence repeat (SSR) markers were employed for molecular characterization of the maize landrace along with five other maize samples which comprised of four improved open pollinated varieties (OPVs). Sequence information of different SSR primers was retrieved from on-line resource maize GDB (http://www.maizegdb.org). Polymerase chain reactions (PCRs) for amplification were carried out with a 10 µl reaction volume with approximately 30 ng of genomic DNA and separated on 3% agarose gel. DNA ladder of 100 bp was run as a reference for determining amplicon size. Polymorphism information content was carried out using Microsoft Excel (online Supplementary Table S1).

Results and discussion

Murali makkai lacks formal crop improvement intervention and has evolved over time adapting to the peculiar hilly conditions of Sikkim. The presence of small-sized cobs with small-sized seeds and prolific habit (5–6 cobs) resemble wild corn (Fig. 1). Data recorded following DUS guidelines revealed distinct traits (Table 1). Tassel showed drooping and strongly curved attitude of lateral branches. Ears were medium in length (10–15 cm) with small diameter (1.93 cm), conico-cylindrical with round flint type grains arranged in a spiral row fashion. Kernels were small with low test weight (87.90 g) and showed excellent popping capacity (>95%). Plants were tall (223.0 cm) and late in maturity (131–135 days). Plants exhibited prolificacy with an average of 5–6 cobs per plant with a mean cob length of 8.33 cm. A mean grain yield of 670 kg/ha was recorded in test plots. PCR amplification with SSR markers amplified a total of 22 amplicons in murali makkai ranging from 100 to 800 bp (online Supplementary Table S2). Primers bnlg1083, umc1353, umc1128, bnlg1017, bnlg2077, umc2298 and umc2373 amplified unique amplicons in murali makkai. SP maize has been reported to be evolved from a common ancestor and similarity with that of pre-chaplote, pre-Nal-Tal and prehistoric corn of Mangelsdorf has been established (Sachan and Sarkar, 1982, 1986). Prolificacy with multiple ears developing from each node is desirable trait for baby corn breeding. A significant study by Prakash *et al.* (2019) on genetic dissection of this landrace revealed quantitative inheritance of prolificacy with non-allelic duplicate epistasis interaction. The same authors reported a novel major Quantitative trait loci (QTL) 'qProl-SP-8.05' conferring prolificacy contributing 31.7% phenotypic variation for the trait utilizing prolific inbred line (Prakash *et al.*, 2021). One of the limitations of utilizing this landrace in maize breeding programme is poor expression of the distinct traits outside the northeastern region (Sharma *et al.*, 2010). Conservation of SP on-farm is a potent strategy where its custodian communities shall be promoted to conserve and expand area under its cultivation through institutional support. In spite of its relatively low yield as compared to other high yielding pop corn hybrids or OPVs, unique trait of prolificacy and adaptation to low input conditions makes it a valuable genetic resource. Considering the current scenario, conservation by use seems to be best strategy for sustainable use of this maize landrace by utilizing it in development of inbred lines, prolific gene pools and population improvement programmes focusing on prolificacy. SSR markers bnlg1083, umc1353, umc1128, bnlg1017, bnlg2077, umc2298 and umc2373

https://doi.org/10.1017/S1479262122000065 Published online by Cambridge University Press

with unique amplicons shared with three other maize populations can be utilized for fingerprinting studies of this landrace. Seed samples of murali makkai have been submitted to ICAR-National Bureau of Plant Genetic Resources, New Delhi for long-term storage for further utilization in maize improvement programmes.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S1479262122000065

Acknowledgements. The authors express deep sense of gratitude to the farmers of Sikkim for providing the maize samples. The authors sincerely thank The Director, ICAR Research Complex for NEH Region, Umiam, Meghalaya for providing necessary facilities for carrying out the study.

References

Dhawan NL (1964) Primitive maize in Sikkim. *Maize Genetics Cooperation Newsletter* **38**, 69–70.

Doyle JJ and Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* **12**, 13–15.

Karuppaiyan R and Avasthe RK (2006) Maize genetic resources in Sikkim, their adaptability and usefulness for hill agriculture. In National Seminar on Physiological and Molecular Approaches for the Improvement of Agricultural, Horticultural and Forestry Crops, at Kerala Agricultural University, Vellanikkara, Thrissur.

Mani VP, Joshi HC and Koranne KD (1987) Preliminary observations on drought tolerance in Sikkim Primitive maize. *Maize Genetics Cooperation Newsletter* **61**, 2.

Prakash Nitish R, Zunjare RU, Muthusamy V, Chand G, Kamboj MC, Bhat JS and Hossain F (2019) Genetic analysis of prolificacy in 'Sikkim Primitive' – a prolific maize (*Zea mays*) landrace of north-eastern Himalaya. *Plant Breeding* **138**, 781–789.

Prakash Nitish R, Zunjare RU, Muthusamy V, Rai M, Kumar A, Guleria SK, Bhatt V, Choudhary J, Chand G, Jaiswal SK, Bhat JS and Hossain F (2021) A novel quantitative trait loci governs prolificacy in 'Sikkim Primitive' – a unique maize (*Zea mays*) landrace of north-eastern Himalaya. *Plant Breeding* **140**, 400–408.

Prasanna BM (2010) Diversity in global maize germplasm: characterization & utilization. *Journal of Biosciences* **37**, 843–855.

Prasanna BM, Sharma L, Wasala SK, Singode A, Kumar R, Guleria, SK, Sekhar JC, Karuppaiyan R, Srinivasan K and Gupta HS (2009) Maize landraces in India – Phenotypic and molecular characterization. In ICAR News, Vol. 15, p. 1.

Sachan JKS and Sarkar KR (1982) Plant type of 'Sikkim Primitives'. *Maize Genetics Cooperation Newsletter* **56**, 122–124.

Sachan JKS and Sarkar KR (1986) Discovery of Sikkim Primitive precursor in the Americas. *Maize Genetics Cooperation Newsletter* **60**, 104–106.

Sharma L, Prasanna BL and Ramesh B (2010) Analysis of phenotypic and micro-satellite based diversity of maize landraces in India, especially from the north east Himalayan region. *Genetica* **138**, 619–631.

Singh B (1977) *Races of Maize in India*. New Delhi, India: Indian Council of Agricultural Research (ICAR), p. 78.

Singode A and Prasanna BM (2010) Analysis of genetic diversity in the north eastern Himalayan (NEH) maize landraces of India using microsatellite markers. *Journal of Plant Biochemistry and Biotechnology* **19**, 33–41.