GRASSROOTS JOURNALS
ISSN 2564-4653 | 03(01) June 2023

AGROBIODIVERSITY & AGROECOLOGY | 03(01) JUNE 2023

Published by The Grassroots Institute (Canada) in partnership with University "Lucian Blaga" from Sibiu (Romania) and Fondacija Alica Banja Luka (Bosnia i Herzegovina). Website: https://grassrootsjournals.org/aa

M – 00346 | Research Article

Identifying Drought Tolerant Finger Millet Landraces for the Hills of Nepal

Krishna Hari Ghimire[1]*, Madhav Prasad Pandey[2], Surya Kanta Ghimire[3], Hira Kaji Manandhar[4], Bal Krishna Joshi[5]
[1]National Agriculture Genetic Resources Center, Nepal Agricultural Research Council, & Agriculture and Forestry University, Rampur, Chitwan, Nepal. Email: ghimirekh@gmail.com | ORCID: https://orcid.org/0000-0002-3393-290X
[2]Department of Genetics and Plant Breeding, Agriculture and Forestry University, Nepal.
Email: mppandey.pb@gmail.com | ORCID: https://orcid.org/0000-0001-8765-3220
[3]Department of Genetics and Plant Breeding, Agriculture and Forestry University, Nepal.
Email: suryaghimire2003@yahoo.com | ORCID: https://orcid.org/0000-0003-2880-3270
[4]Department of Plant Pathology, Agriculture and Forestry University, Nepal.
Email: hirakaji@gmail.com | ORCID: https://orcid.org/0000-0001-7243-0841
[5]National Agriculture Genetic Resources Center, Nepal Agricultural Research Council, Nepal.
Email: joshibalak@yahoo.com | ORCID: https://orcid.org/0000-0002-7848-5824
* *Corresponding author*

How to cite this paper: Ghimire, K.H., Pandey, M.P., Ghimire, S.K., Manandhar, H.K. and Joshi, B.K. (2023). Identifying Drought Tolerant Finger Millet Landraces for the Hills of Nepal. *Agrobiodiversity & Agroecology*, 03(01): 1-27. Doi: https://doi.org/10.33002/aa030101

Received: 05 January 2023
Reviewed: 15 March 2023
Accepted: 28 March 2023
Published: 30 June 2023
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Abstract
Finger millet is in the fourth rank among the major cereals in Nepal after rice, maize and wheat. Since it is cultivated in rainfed conditions, drought is one of the vital production constraints for this crop. With the objective of enhancing the utilization of native landraces conserved *ex situ* in genebank, 300 finger millet accessions were evaluated under drought stress and normal conditions at Khumaltar (1,360 masl), Lalitpur, Nepal during 2017 and 2018. In both the years, genotypes were significantly different for the yield and other agronomic characters. Eight different drought tolerant indices namely drought resistance index, geometric mean productivity, harmonic mean, mean productivity, stress susceptibility index, tolerance, yield index and yield stability index for each genotype were calculated using grain yield data recorded from the stress (Yds) and non-stress (Yns) experiment. Majority of the landraces were grouped under moderately susceptible group in both the years followed by moderately tolerant and susceptible group based on all the drought tolerant indices (DTIs). Five landraces, namely NGRC4849, NGRC6487, NGRC04852, NGRC03491 and NGRC6490 with average grain yield of 4670, 3624, 3426, 3191 and 3132 kg/ha, respectively, were identified as stable drought tolerant landraces compared to the released varieties. These landraces could be the potential sources of drought tolerance for finger millet improvement program for the mid-hills of Nepal.

Keywords
Drought tolerance indices; Finger millet; Grain yield; Native landraces; Rainfed farming

1. Introduction

Finger millet [*Eleusine coracana* (L.) Gaertn., 2n=4x=36] is grown by the East-African and South-Asian farmers for human consumption as well as livestock fodder. It was domesticated in Eastern Africa around 3000 BC and later introduced to the Indian subcontinent around 1000 BC, as reported by Hilu, De Wet and Harlan (1979) and Upadhyaya *et al.* (2006). Although, it is the fourth important crop of the millet group after sorghum, pearl millet and foxtail millet (Upadhyaya, Gowda and Reddy, 2007), its area and production are not precisely available in many countries since its production statistics is

jointly presented with other crops of the millet group (Upadhyaya *et al.*, 2010). The global finger millet area has been reported as 3.8 million ha, mainly in Uganda, Tanzania, Kenya, Ethiopia, Rwanda and Somalia in Africa, as well as India, Myanmar, Nepal, Sri Lanka, China and Japan in Asia (Bora, 2013; Hittalmani *et al.*, 2017; Kumar *et al.*, 2016; Vetriventhan *et al.*, 2016). It is cultivated in wider altitude ranges from the sea level in India (Upadhyaya *et al.*, 2006) to high mountains (3,130 masl) of Nepal (Bastola *et al.*, 2015; Gaihre, Gauchan and Timsina, 2021; Ghimire *et al.*, 2022). It is a climate resilient crop grown in marginal soils with minimal inputs (Goron *et al.*, 2015). Its grain is considered as nutritionally rich compared to major cereals, with high levels of protein, carbohydrate, dietary fiber, ash, calcium, iron, zinc, polyphenols and soluble fiber (Chandra *et al.*, 2016; Devi *et al.*, 2014; Krishna, Reddy and Kumar, 2021; Nakarani *et al.*, 2020). Since it is a hardy crop grown in marginal soils, it is considered as the future smart crop due to its high nutritional richness with great potentiality to cope with the problem of food and nutrition insecurity in the changing climatic circumstances (Ghimire *et al.*, 2020). Finger millet is called as *kodo* in Nepali language. Its flour has been commonly used for human food, straw is used as quality fodder for livestock, and whole grains are fermented for making alcoholic beverages (Ghimire *et al.*, 2020). The green as well as dry straw of finger millet is very good animal fodder, which contains about 61% total digestible nutrients (Wafula *et al.*, 2017). The demand of this crop by urban health-conscious consumers is increasing rapidly in recent years. It is nowadays emerging as a crucial element of agricultural tourism in Nepalese homestays serving the traditional recipes such as *selroti* (a ring bread), *dhindo* (thick porridge) and *raksi* (high quality wine) (Ghimire *et al.*, 2017; Gaihre, Gauchan and Timsina, 2021; Joshi, Joshi and Ghimire, 2020).

Finger millet played a vital role in Nepalese economy since it became the fourth important crop of the country after major cereals like rice, maize and wheat (Ghimire *et al.*, 2020). It is cultivated in 265,401 ha area of Nepal producing 326,443 ton grains with average productivity of 1.23 t/ha (MoALD, 2022). More than 90% of the total finger millet produced in the country comes from mid-hill districts where it is totally a rainfed crop (Ghimire *et al.*, 2017; MoALD, 2022). Although, National Genebank is holding 1,055 finger millet accessions in long-term conservation (Genebank, 2022), their utilization in breeding is very less. Breeding the crop varieties is always focused on non-stress environment compared to the stress environment because it is easier to show the research results. However, breeding a crop like finger millet for abiotic stress tolerance is always kept in shadow by national and international research systems (Ghimire *et al.*, 2020). There are only 5 varieties (Okhle-1, Dalle-1, Kabre kodo-1, Kabre kodo-2, Shailung kodo-1) released and one variety (Rato kodo) registered in Nepal during the last five decades (Joshi *et al.*, 2017; Ghimire *et al.*, 2017; SQCC, 2022), but none of these varieties are drought tolerant. Drought is condition of very low soil moisture leading to reduced crop yield (Krishna, Reddy and Kumar, 2021). It is one of the most significant abiotic constraints to plant growth that limits the agricultural productivity worldwide. It is recurrent but unpredictable phenomenon hindering the crop production in rainfed agriculture. Finger millet is considered as a drought resilient crop when compared to other major cereals (Vetriventhan *et al.*, 2016). However, its growth can still be adversely affected by both intermittent and terminal drought stresses in the African and South Asian environments (Kumar *et al.*, 1987;

Mwangoe, Kimurto and Ojwang, 2022). This is because finger millet is mainly grown by subsistence farmers under rainfed agriculture, which increases the vulnerability of yield loss due to drought stress. Different finger millet genotypes express different degree of tolerance as the crop is grown totally under rainfed agroecology in Nepal. The rainfall pattern in the semi-arid conditions of central to far-western hills is unpredictable and intermittent drought stress occurs frequently during all the crop growth stages. In semi-arid as well as arid environments where millets are predominantly grown, drought is the major abiotic factor for the yield loss (Ghimire *et al.*, 2020; Tadele, 2016). Farmers from Lumbini, Karnali and Far-western provinces of Nepal are experiencing severe drought in recurring years during finger millet growing season. It has been reported that the reproductive and gain filling stages are the most sensitive to drought stress in finger millet, causing the significant reduction of grain yield (Bhavya *et al.*, 2022; Talwar *et al.*, 2020).

Grain yield under drought is a complex trait to evaluate because of its low heritability (Sharma *et al.*, 2022). The yield data under stress and non-stress are useful to identify stable genotypes tolerant to drought stress (Clarke, De Pauw and Townley-Smith, 1992; Mohammadi *et al.*, 2010; Nouri *et al.*, 2011). There are several methods proposed by various researchers to determine different drought tolerance indices (DTIs) to identify drought tolerant genotypes in different crops (Ferede *et al.*, 2020). Among them, drought resistance index (DRI), geometric mean productivity (GMP), harmonic mean (HM), mean productivity (MP), stress susceptibility index (SSI), tolerance (TOL), yield index (YI) and yield stability index (YSI) are some of the commonly used DTIs (Antre *et al.*, 2021; Bhavya *et al.*, 2022; Ferede *et al.*, 2020; Mardeh *et al.*, 2006; Mau *et al.*, 2019; Menezes *et al.*, 2014). This research was aimed to identify finger millet landraces tolerant to reproductive stage drought stress using different DTIs by evaluating native finger millet landraces of Nepal under drought imposed and under normal field conditions over two consecutive years of 2017 and 2018.

2. Methodology

2.1. Plant Materials

This research used 300 native finger millet genotypes including 295 landraces and five released varieties. These diverse genetic resources were collected from 54 districts of six provinces and conserved at National Agriculture Genetic Resources Center (NAGRC) commonly known as Nepal Genebank. District-wise number of landraces has been given in figure 1. Detail passport information (local names, collection districts and geo-cordinates) of these genotypes has been provided in Supplementary Table S1.

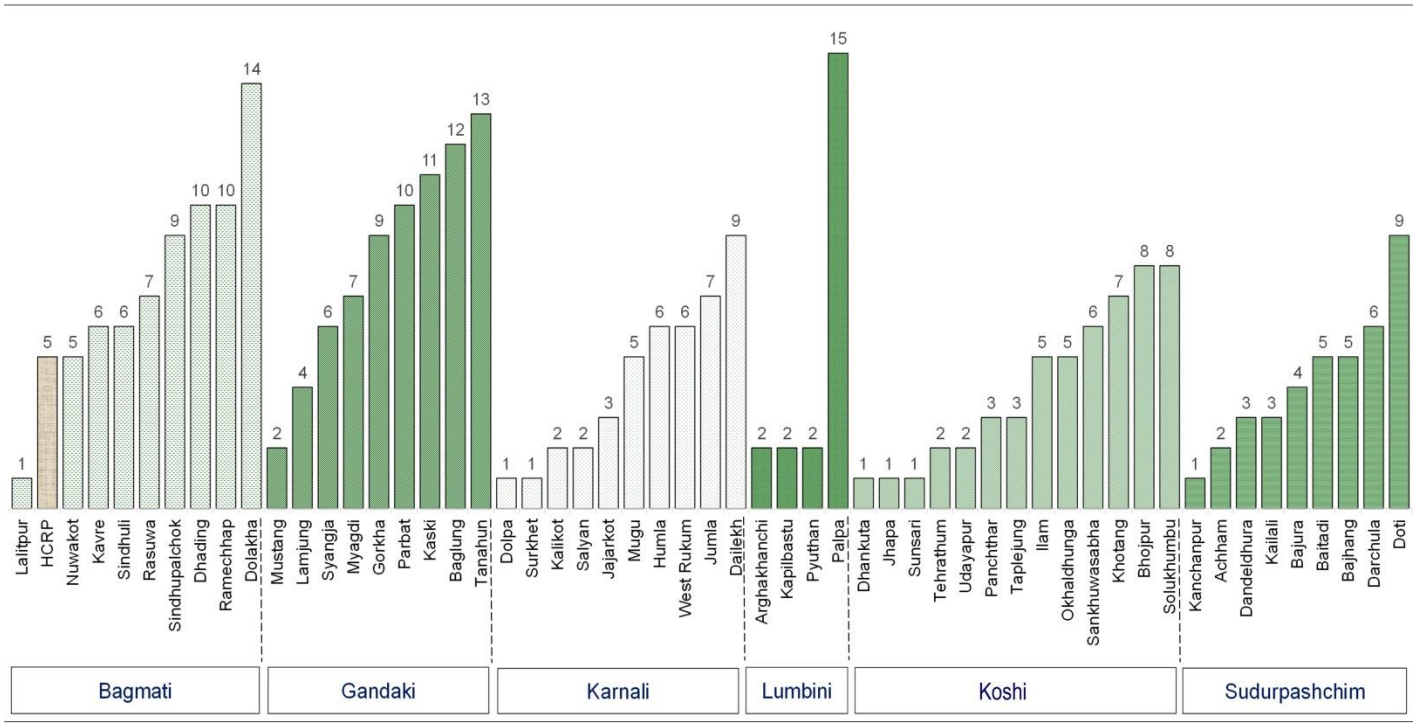

Figure 1: District and province-wise frequency of finger millet genotypes used in the experiment.

2.2. Experiment Site

This experiment was conducted in Khumaltar, Lalitpur, Nepal located at an altitude of 1,360 meter above sea level, north latitude of 27.65° and east longitude of 85.32° representing mid-hill environment of Nepal. The site has fluvial non-calcareous slightly acidic soil with 53.4% sand, 16.5% clay, 2.5% organic matter and pH value of 6.2 (https://soil.narc.gov.np). Data on monthly total rainfall (mm), relative humidity (average, %) and temperature (°C) of the site during finger millet season of both the years has been summarized in table 1.

Table 1: Weather data of experiment site during finger millet growing period (May to November) of 2017 and 2018

Month	*Relative humidity (%)*				*Minimum-maximum temperature (°C)*				*Rainfall (mm)*	
	2017		*2018*		*2017*		*2018*		*2017*	*2018*
	ns	ds	ns	ds	ns	ds	ns	ds	ns	ns
May	76	75	73	73	16–28	17–31	17–27	17–31	150	60
June	81	80	81	82	20–29	21–33	20–29	20–33	200	128
July	82	82	84	85	21–28	21–33	21–28	22–33	216	387
August	81	82	82	82	21–28	21–33	21–28	21–33	266	322
September	79	78	80	81	20–29	21–33	19–28	20–33	103	53
October	79	79	69	70	16–28	16–31	12–26	12–30	1	0
November	75	75	72	72	8–24	9–28	7–23	7–25	0	0

Note: ds=drought stress (under white transparent plastic tunnel), ns=non-stress (open field).

2.3. General Methodology

The authors established two parallel set of experiments: one under plastic tunnel creating drought stress (ds) condition, and another in the open field or non-stress (ns) condition. White transparent plastic roof was provided in ds experiment to prevent from the rainfall. For both the experiments, direct seeding was done in raised beds on 17 June 2017 and 8 July 2018. A total of 300 entries were evaluated in alpha lattice design[1] with two replications and 15 blocks. Each plot comprised 10 plants (single row of 1 m length) in ds and 20 plants (single row of 2 m length) in ns with row spacing of 25 cm. For both ds and ns experiments, 20 kg N, 10 kg P_2O_5 and 10 kg K_2O per hectare were applied at basal doses, whereas 20 kg/ha N was top-dressed after six weeks of sowing. Manual weeding followed by thinning was done after four weeks of seeding to maintain 10 cm spacing between plants within rows. Sprinkler irrigation was provided at an interval of two weeks till the initiation of flowering in ds but no irrigation was provided in ns field. Soil moisture was recorded from 10 cm depth in a day interval from both ds and ns fields (Ghimire *et al.*, 2020).

2.4. Data Recording and Analysis

Agronomic data on various traits such as days to 50% flowering, days to 80% maturity, grain yield (kg/ha, adjusted to 14% grain moisture) and straw yield (ton/ha after week-sun-drying) were recorded from ds and ns experiments based on whole plot, whereas number of fingers per head was taken from five randomly selected plants. Data were subjected to analysis of variance (ANOVA) and different variability components (mean, standard error of mean, minimum, maximum, probability value, broad sense heritability (h^2bs) and genetic advance with percentage of mean (GAM) were calculated using 'variability' package (Popat, Patel and Parmar, 2020) of R statistical software (R Core Team, 2020). Various drought tolerance indices (DTIs) were calculated for each genotype using Excel worksheet while referring the relationship of mean grain yield under ds and ns (Table 2). Overall mean (μ) and standard deviation (σ) were calculated for each DTIs. Genotypes were grouped in five reaction classes based on DTI values. Higher drought tolerance is considered when the DTI value is higher in case of DRI, GMP, HM, MP, SSI, YI and YSI. Thus, genotype is grouped as tolerant (T) if DTI is above μ+2σ, moderately tolerant (MT) if DTI=μ+σ to μ+2σ, moderately susceptible (MS) if DTI=μ-σ to μ+σ, susceptible (S) if DTI=μ-2σ to μ-σ and highly susceptible (HS) if DTI is below μ-2σ (Antre *et al.*, 2021). This is just reverse in case of TOL because the genotype showing greater TOL value means there is higher yield reduction and higher drought sensitivity (Mardeh *et al.*, 2006).

[1] Alpha lattice design is a replicated simple experimental design like randomized complete block design (RCBD), but is efficient with large number of treatments where all treatments cannot be adjusted in a homogenous block or in single terraces especially in hills, thus called as an incomplete block design (Akinwale *et al.*, 2021) which is used for the experiments under rainfed conditions, when there were higher CV (%) with RCBD, and gives higher efficiency than the RCBD (Yau *et al.*, 1997).

Table 2: Different drought tolerance indices (DTIs) used in the study

DTIs	*Formula*	*Reference*
Drought resistance index (DRI)	$DRI = Yds \times \left(\frac{Yds}{Yns}\right) / \bar{Y}ns$	Lan (1998)
Geometric mean productivity (GMP)	$GMP = (Yns \times Yds)^{0.5}$	Fernandez (1992); Schneider *et al.* (1997)
Harmonic mean (HM)	$HM = \{2(Yns \times Yds)/(Yns + Yds)\}$	Bidinger, Mahalakshmi and Rao (1987); Jafari, Paknejad and Al-Ahamadi (2012)
Mean productivity (MP)	$MP = (Yds + Yns)/2$	Rosielle and Hamblin (1981)
Stress susceptibility index (SSI)	$SSI = \{1 - (Yds/Yns)\} / \{1 - (\bar{Y}ds/\bar{Y}ns)\}$	Fischer and Maurer (1978)
Tolerance (TOL)	$TOL = Yns - Yds$	Rosielle and Hamblin (1981); Hossain *et al.* (1990)
Yield index (YI)	$YI = Yds/\bar{Y}ds$	Gavuzzi *et al.* (1997)
Yield stability index (YSI)	$YSI = Yds/Yns$	Bouslama and Schapaugh (1984)

Note: Yds=individual genotype yield under drought, Yns= individual genotype yield under non-stress, $\bar{Y}ds$=average yield of the entries under drought, $\bar{Y}ns$=average yield of the entries under non-stress

3. Results

3.1 Soil Moisture Depletion under ds and ns Field

The experiment established under transparent white plastic tunnel preventing from rainfall is called drought stress (ds), whereas experiment in the open field is called as non-stress (ns) experiment. Soil moisture was recorded from 10 random points of both ds and ns experiments at 10 cm depth using soil moisture measuring device. Average soil moisture on every alternate day from mid-August to the first week of November during 2017 and 2018 is plotted in figure 2. Soil moisture depleted from 25% on 16 August to 14% on 10 November of 2017 in ns field conditions, whereas this depletion was from 17% on 16 August to 5% on 10 November of 2017 in ds field conditions. Similarly, during 2018, soil moisture reduction was from 24% on 15 August to 10% on 9 November in ns field, and the same was from 17% on 15 August to 6% on 9 November in ds field conditions. There was a difference of about 11-17% between soil moisture in ns and ds field conditions during the first year (i.e. 2017), but this difference was lower (3-10%) during the second year (i.e. 2018). The crop under ds conditions showed more stress in 2017 than in 2018.

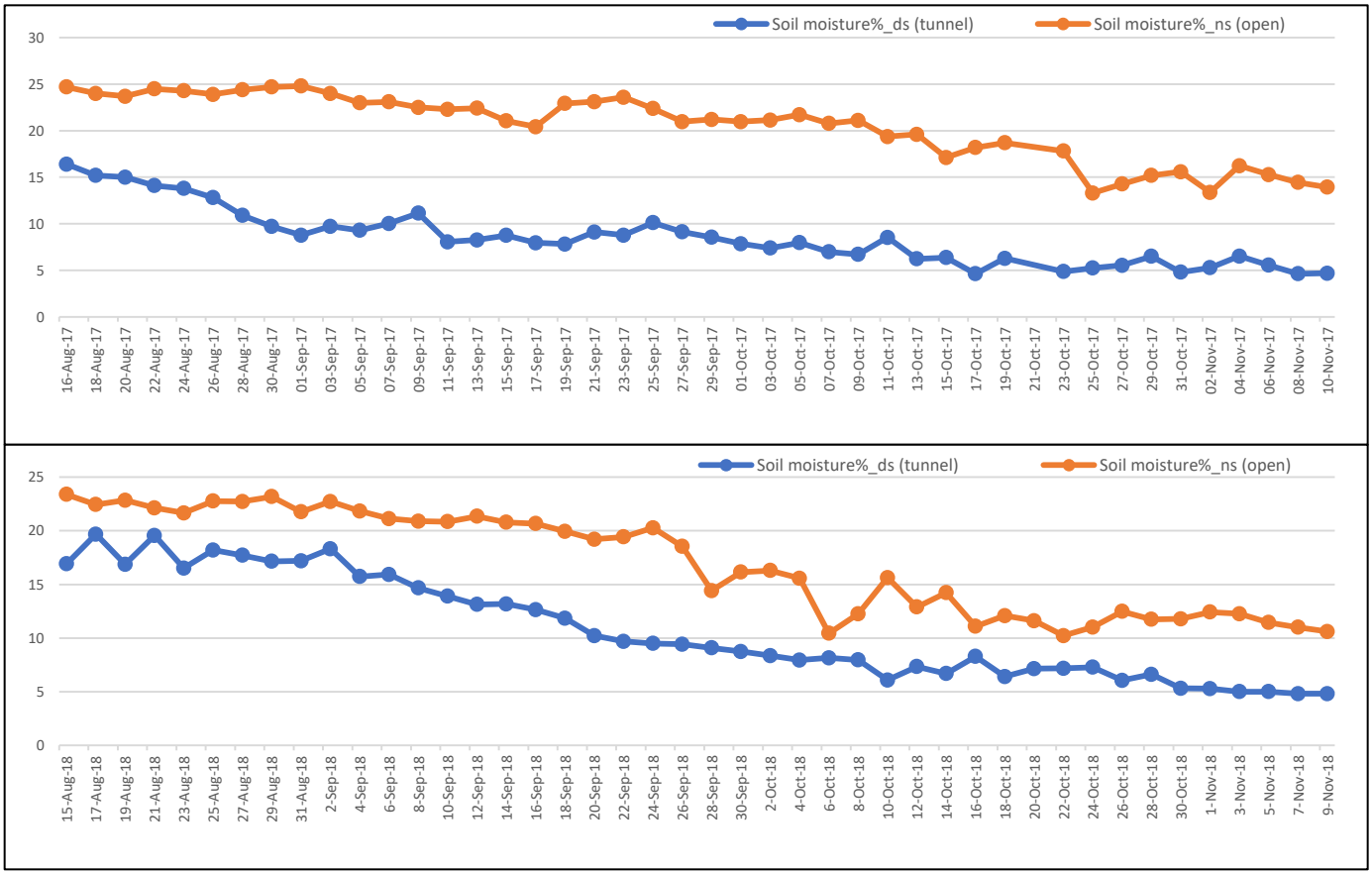

Figure 2: Average (n=10) moisture (%) of experimental soils at the depth of 10 cm during vegetative to maturity stage of the finger millet in 2017 (top) and 2018 (bottom)

3.2. Variability among Genotypes for Agronomic Traits

Wider variation was recorded among the genotypes under ds and ns conditions for seven different agronomic traits in 2017 and 2018 (Table 3). Grain yield under ds ranged from 39-7,263 kg/ha in 2017 and 81-5,403 kg/ha in 2018; whereas this range was from 137-7,514 kg/ha and 146-6,946 kg/ha, respectively, for 2017 and 2018 under ns conditions. Genotypes differed significantly for days to flowering, number of fingers and grain yield under ds and ns experiments in 2017 as well as in 2018. The genotypes significantly differed for straw yield under ds condition. Genotypes were not significantly different for plant height under ds in both the years, but the difference was significant for the plant height under ns during both the years. The difference among the genotypes was not significant for the root length under ds during both the years. Moderately high to very high heritability (h^2bs) was observed for days to flowering (62.1-93.1%) under ds and ns conditions during both the years, but h^2bs for grain yield and other traits were found low to medium (11.8-55%) in ds and ns conditions during both the years. High GAM was observed for days to flowering (20.1%-31.2%) under ds (2017 and 2018) and ns (2017 and 2018), for grain yield (60.2-80.7%) under ds (2017), and ns (2017 and 2018), as well as for straw yield (31.2-38.4%) under ds (2017 and 2018). Mean grain yield under drought was 989 and 1,529 kg/ha in 2017 and 2018, respectively. Relative mean grain yield reduction due to drought was 31.65% in

2017 and 1.86% in 2018, suggesting very low impact of drought stress in the second year. This is due to the lower soil moisture difference between ns field and ds field in the second year.

Table 3: Variance components of finger millet genotypes evaluated under drought stress and non-stress conditions for different agronomic traits over two years

Trait	*Year*	*Env*	*Mean ± SE*	*Range*	*P-value*	*h^2bs (%)*	*GAM (%)*
DTF	2017	ds	89 ± 5.86	48-135	0.000	71.2	25.6
		ns	96 ± 2.88	48-123	0.000	93.1	31.2
	2018	ds	87 ± 5.93	50-140	0.000	62.1	20.1
		ns	102 ± 2.98	55-130	0.000	91.3	26.4
EE	2017	ds	8.1 ± 1.93	1.6-19.6	0.263	-	-
		ns	12.1 ± 1.44	2.6-19.8	0.000	38.6	17.2
	2018	ds	11.8 ± 2.63	2.4-26.4	0.019	11.9	8.24
		ns	10.2 ± 2.42	2.7-23.4	0.008	14.0	10.4
FPH	2017	ds	7.5 ± 1.27	2.0-15.0	0.000	18.6	10.1
		ns	6.6 ± 0.75	2.0-11.8	0.000	51.1	24.3
	2018	ds	9.2 ± 1.16	4.0-14.8	0.000	24.0	10.1
		ns	7.3 ± 0.88	3.4-11.0	0.020	11.8	4.42
GY	2017	ds	989 ± 564.9	39-7263	0.000	30.2	60.2
		ns	1447 ± 489.7	137-7514	0.000	55.0	80.7
	2018	ds	1529 ± 498.2	81-5403	0.013	12.9	13.1
		ns	1558 ± 470.8	146-6946	0.000	53.0	72.7
PH	2017	ds	68 ± 10.66	29-103	0.119	-	-
		ns	81 ± 7.43	32-130	0.000	46.5	17.0
	2018	ds	122 ± 12.70	53-171	0.795	-	-
		ns	110 ± 8.85	50-151	0.000	34.3	10.0
RL	2017	ds	15.5 ± 2.26	7.5-27.5	0.115	-	-
	2018	ds	15.2 ± 2.17	7.4-27.8	0.198	-	-
SY	2017	ds	7.9 ± 1.37	1.9-13.7	0.000	52.2	38.4
	2018	ds	9.3 ± 1.62	1.6-16.2	0.000	45.6	31.2

Note: DTF = Days to 50% flowering, EE = Ear exsertion, FPH = Number of fingers per head, GY = Grain yield (kg/ha), PH = Plant height (cm), RL = Root length (cm), SY = Straw yield (t/ha), ds = Stress, ns = Non-stress, SE = Standard error of mean, h^2bs = Heritability in broad sense, GAM = Genetic advance with percentage of mean

3.3. Variability among Genotypes for DTIs

Grain yield under ds and ns field conditions was used to calculate different drought tolerant indices of each genotype. High variability was observed among genotypes for all the eight DTIs namely DRI, GMP, HM, MP, SSI, TOL, YI and YSI in both the years (Table 4). Genotype with lower TOL value is preferable since it calculates the yield difference between ns and ds fields, but higher value is desirable for rest of the DTIs.

Table 4: Mean and range of eight various drought tolerant indices over two years

DTI	*Year*	*Mean*	*SE*	*Minimum*	*Maximum*
DRI	2017	0.87	0.11	0.00	26.82
	2018	1.61	0.10	0.01	13.59
GMP	2017	1117.1	40.8	100.2	6427.9
	2018	1430.3	31.9	335.8	4227.7
HM	2017	1037.6	40.7	99.6	6419.9
	2018	1374.7	31.6	275.2	4101.8
MP	2017	1218.5	41.6	100.8	6436.0
	2018	1493.9	32.7	343.7	4546.5
SSI	2017	0.09	0.29	-68.53	3.05
	2018	7.53	1.21	-17.74	141.84
TOL	2017	460.8	52.2	-2137	4123
	2018	73.4	51.8	-2185	3931
YI	2017	1.00	0.05	0.05	6.84
	2018	1.00	0.02	0.10	2.43
YSI	2017	0.97	0.09	0.03	22.79
	2018	1.38	0.06	0.11	8.14

Based on the DTI values, all 300 genotypes were divided into five reaction groups namely tolerant, moderately tolerant, moderately susceptible, susceptible and highly susceptible. Number of genotypes in each group for all the DTIs during both the years has been presented in table 5. Large numbers of the genotypes were grouped under moderately susceptible group based on DRI (95% and 90%), GMP (84% and 74%), HM (83% and 72%), MP (83% and 78%), SSI (96% and 88%), TOL (70% and 74%), YI (85% and 72%) and GMP (96% and 88%) in 2017 and 2018, respectively. During 2017, 10 genotypes each in GMP and MP, 9 each in HM and YI, 8 in DRI, 7 in TOL and 5 genotypes in GMP were found as tolerant. Similarly, during 2018, maximum of 15 genotypes in SSI, 14 in YSI, 12 in MP, 11 each in DRI and GMP, 9 each in HM and TOL and 7 in YI were grouped as tolerant genotypes. None of the genotypes were found as highly susceptible for 6 out of 8 DTIs except SSI and TOL in 2017, whereas 12 genotypes for TOL, 5 for YI and only 1 each for HM and MP were found as highly susceptible in 2018.

Table 5: Number of genotypes under different reaction groups and drought tolerant indices over two years

Reaction level	*Year*	*DRI*	*GMP*	*HM*	*MP*	*SSI*	*TOL*	*YI*	*YSI*
Tolerant	2017	8	10	9	10	0	7	9	5
	2018	11	11	9	12	15	4	7	14
Moderately tolerant	2017	9	16	22	19	0	37	22	9
	2018	22	31	36	25	17	36	32	18
Moderately susceptible	2017	281	250	247	244	284	207	251	284
	2018	265	219	214	230	260	219	213	260
Susceptible	2017	0	22	20	25	9	38	16	0
	2018	0	37	38	30	6	27	41	6
Highly susceptible	2017	0	0	0	0	5	9	0	0
	2018	0	0	1	1	0	12	5	0

3.4. Stable Drought Tolerant and Susceptible Genotypes

Genotypes showing tolerant reaction across DTIs and over two years were selected as stable drought tolerant genotypes, whereas genotypes showing susceptible reaction across DTIs and over two years were found as stable susceptible genotypes for drought stress. Five tolerant and five susceptible genotypes with their contrasting reaction levels in each DTI and year have been presented in table 6. Landraces namely NGRC04849, NGRC06487, NGRC04852, NGRC03491 and NGRC06490 showed tolerant to moderately tolerant reaction in at least 5 out of 8 DTIs during both the years. In contrast, 5 genotypes namely NGRC06485, NGRC04857, NGRC01634, NGRC03503 and NGRC03540 showed moderately susceptible to highly susceptible reactions for at least 7 out of 8 DTIs during both the years.

Table 6: Stable drought tolerant and susceptible finger millet landraces based on drought tolerant indices over two years

Genotype	*Year*	*DRI*	*GMP*	*HM*	*MP*	*SSI*	*TOL*	*YI*	*YSI*
NGRC04849	2017	T	T	T	T			T	
	2018		T	T	T			MT	
NGRC06487	2017	T	T	T	T			T	
	2018		T	T	T			MT	
NGRC04852	2017	MT	T	T	T			T	
	2018		MT	MT	MT	MT			MT
NGRC03491	2017	MT	MT	MT	MT			T	
	2018	T	MT	MT	MT	MT	T	T	MT
NGRC06490	2017		T	T	T		T	T	
	2018		T	T	T	MT		T	MT
NGRC06485	2017	MS	S	S	S	HS	S	MS	
	2018	MS	S	S	S	MS	MS	MS	MS
NGRC04857	2017	MS	S	S	S	S	MS	MS	
	2018	MS	S	S	S	MS	MS	S	MS
NGRC01634	2017	MS	S	S	S	MS	MS	S	MS
	2018	MS	S	S	S	S	S	HS	S
NGRC03502	2017	MS	S	S	S	MS	MS	S	MS
	2018	MS	S	S	S	MS	MS	HS	MS
NGRC03540	2017	MS	S	S	S	MS	MS	S	MS
	2018	MS	S	HS	S	S	S	HS	S

Mean grain yield performance combined over years of tolerant and susceptible landraces together with 5 released varieties has been presented in figure 3. The highest mean grain yield was produced by NGRC04849 (4,670 kg/ha) followed by NGRC06487 (3,624 kg/ha), NGRC04852 (3,426 kg/ha), NGRC03491 (3,191 kg/ha) and NGRC06490 (3,132 kg/ha). These stable tolerant genotypes produced 149% to 271% higher grain yield over the grand mean (1,259 kg/ha) under drought stress. The susceptible landraces produced grain yield from 172 to 821 kg/ha, which was 35% to 86% lower as compared to grand mean. Four out of five released varieties produced average grain yield higher than grand mean, but much lower than stable tolerant landraces.

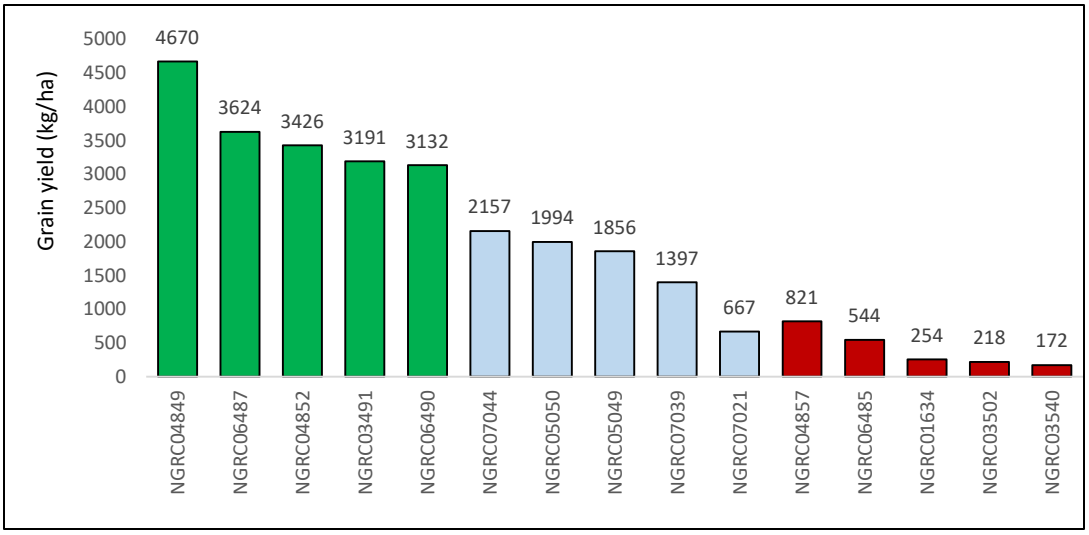

Figure 3: Mean grain yield performance of tolerant and susceptible landraces with five released varieties of finger millet

4. Discussion

Genebanks conserve genetically diverse genetic resources, which can be utilized to develop high-yielding and stable crop varieties tolerant to various biotic and abiotic stresses, and that contribute to global food security. Finger millet landraces exhibit significant variation in grain yield under different agroecological conditions, but their potential for breeding programs was not efficiently utilized in developing countries, including Nepal, due to limited attention paid to their characterization, evaluation, and pre-breeding activities. Despite the diverse genetic resources conserved in genebanks, less than 10% has been utilized in crop breeding. This is primarily due to insufficient evaluation work especially for the complicated traits like yield under drought (Hodgkin *et al.*, 2003; Nguyen and Norton, 2020). Breeding the finger millet varieties for drought stress tolerance is always at low priority in Nepal, hence not a single variety is released as drought tolerant variety suitable for the dry areas of mid hills (SQCC, 2022).

The weather condition of the natural field is always unpredictable, and rainfall is beyond the control of researchers. To avoid undesirable but unexpected rainfall in the drought screening field, controlled structure with roofing of white transparent plastic sheet was used in this experiment. There was a difference of about 11-17% in soil moisture between under stress and non-stress field conditions during the first year; but this difference was only 3-10% during the second year. The crop under drought showed more stress in 2017 than in 2018. It was reflected in mean grain yield under stress, i.e. 989 kg/ha in 2017 and 1529 kg/ha in 2018. Degree of drought stress was estimated with relative yield reduction under drought compared to the yield under non-stress field conditions. Relative reduction in mean grain yield due to drought stress was 31.65% in 2017 was and 1.86% in 2018, suggesting very low impact of drought stress in the second year. This is due to the lower soil moisture difference between non-stress and stress field conditions in the second year. The yield reduction under drought was not uniform in both the years, suggesting that the

genotypes do respond differently according to the extent of drought stress (Antre *et al.*, 2021). It is found that this type of controlled structure was effective for screening genotypes under drought stress to avoid unpredictable rainfall. However, high infestation of insects mainly aphids and stem borers was evident due to dryness in such fields. The maximum temperature under ds field was 3-5°C higher compared to ns field. There was very high fluctuation in intra-day relative humidity (25-99%) as per the temperature fluctuation under ds field. The effect of light intensity under the plastic structure was not taken care of.

Singh (2001) explained greater than 80% the heritability values as very high, 60-79% the heritability values as moderately high, 40-59% the heritability values as medium, and less than 40% the heritability values as low. According to this delineation, moderately high to very high level of h^2bs for required days to flowering are observed. Medium h^2bs for grain yield under ns conditions and low h^2bs under ds conditions during both the years indicated the inheritance of grain yield mostly controlled by environmental variation. Previous reports by Anuradha *et al.* (2020); Bezaweletaw *et al.* (2006); Lule *et al.* (2012); Wolie, Dessalegn and Belete (2023) showed comparatively higher heritability in finger millet as compared to our estimates. Lower estimates of h^2bs in the study may be due to smaller plot size and single location (Teklu, Kebede and Gebremichael, 2014). This suggests the need of evaluation in multiple locations with larger plot size for effective selection of genotypes. Genetic advance as percentage of mean (GAM) values less than 10% are considered as low, 10-20% as moderate and more than 20% as high (Johnson, Robinson and Comstock, 1955). Based on this demarcation, the high genetic advance was estimated for days to flowering under ds and ns conditions in both the years. Similarly, grain yield under ds (2017), ns (2017 and 2018) and straw yield under ds (2017) showed high GAM. Sharma *et al.* (2022) reported higher h^2bs and GAM in finger millet as compared to the estimates of this study. Significant difference among the genotypes was observed for days to flowering, ear exertions, number of fingers per head, grain yield and straw yield, but the difference was not significant for plant height and root length under ds conditions. Similar results have reported that finger millet genotypes were not significantly different for root length under drought (Aparna and Bhargavi, 2017) and grain yield under drought stress was not correlated with root length (Simbagije, 2016). In contrast, morphological traits such as shoot length and root length are important traits under drought stress conditions (Mukami *et al.*, 2019; Murtaza *et al.*, 2016; Mwangoe, Kimurto and Ojwang, 2022).

Genotypes showing significant variability in grain yield under ds conditions suggest genotypic tolerance to drought stress. It is difficult to make conclusion just by looking at the yield performance under stress condition; thus, the comparative yield performance of genotypes under drought stress and non-stress conditions are useful to identify stable drought tolerant genotypes (Clarke, De Pauw and Townley-Smith, 1992; Mohammadi *et al.*, 2010; Moosavi *et al.*, 2008; Naghavi, Aboughadareh and Khalili, 2013; Nouri *et al.*, 2011). Genotypes displaying high yield variation under drought stress and non-stress conditions cannot be measured as a stable drought tolerant genotype even if it yielded high under drought (Ali and El-Sadek, 2016). There are several methods proposed by various researchers to determine different DTIs in different crops to identify stable genotypes tolerant to drought. Among them, DRI, GMP, HM, MP, SSI, TOL, YI and YSI are some of the commonly used DTIs (Antre *et al.*, 2021; Bhavya *et al.*, 2022; Darkwa *et al.*, 2016; Ferede *et al.*, 2020; Mardeh *et al.*,

2006; Mau *et al.*, 2019; Menezes *et al.*, 2014; Naghavi, Aboughadareh and Khalili, 2013). High values of DRI, SSI, YI and YSI confirmed drought tolerance in different crops whereas high value of GMP, HM and MP confirmed the stable genotypes giving high yield at various levels of drought stress as well as non-stress condition (Ferede *et al.*, 2020; Ghasemi and Farshandfar, 2015; Mohammadi *et al.*, 2010; Naghavi, Aboughadareh and Khalili, 2013; Yousefi, 2015). In contrary to other DTIs, lower TOL value is desirable to be concluded as genotypes less sensitive to drought stress (Clarke, De Pauw and Townley-Smith, 1992; Mardeh *et al.*, 2006; Rosielle and Hamblin, 1981). However, the selection only based on low TOL value was not effective to detect high yielding genotypes (Rizza *et al.*, 2004). Stability in grain yield performance under both the ds and ns conditions is important to detect genotypes with stable drought tolerance (Mardeh *et al.*, 2006). Based on the reaction levels exhibited by the genotypes across DTIs, five landraces namely NGRC04849, NGRC06487, NGRC04852, NGRC03491 and NGRC06490 were identified as stable tolerant landraces under drought conditions in this study because they showed tolerant to moderately tolerant reaction for at least 5 out of 8 DTIs during both the years. These stable landraces should be utilized in different ways: as donors in breeding program, further evaluation in larger plots at multiple locations, bringing into formal seed system after registration as a variety.

5. Conclusion

Wide variation was observed within Nepalese finger millet genotypes for agronomic traits under drought. Based on grain yield under stress and non-stress experiments, eight drought tolerant indices were used to identify stable drought tolerant landraces. Based on these indices, five promising landraces are identified, namely NGRC4849 from Rukum, NGRC6487 from Sindhuli, NGRC04852 from Sindhupalchok, NGRC03491 from Dolakha and NGRC6490 from Sindhuli district. These landraces have average grain yield ranging from 3,100 to 4,600 kg/ha showing stability compared to the released varieties under both drought and non-stress condition of mid-hill environments. This finding could provide strong avenues to enhance the use of native finger millet landraces and to accelerate the breeding process of this future smart nutrient-dense cereal. Since none of the finger millet varieties released so far is recommended for drought-prone areas, these selected landraces are being tested in larger plots under national coordinated varietal trials by Hill Crops Research Program (HCRP) throughout the country. Besides, these landraces could be listed into the national gazette as a variety, deployed to the analogous hilly environments to enrich the varietal options enhancing food security of the country.

6. Acknowledgments

This research was funded by UNEP/GEF in coordination with Bioversity International. We acknowledge the logistic support received from Agriculture and Forestry University (AFU), Nepal Agricultural Research Council (NARC) and National Agriculture Genetic Resources Center (NAGRC) for the field experiments. We extend our sincere gratitude to all farmers who provided seeds of these landraces.

7. Supplementary Table S1

Passport details of 300 finger millet genotypes received from National Agriculture Genetic Resources Center (Genebank), Nepal used in this research. Supplementary Table S1 is appended as zip file.

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Authors' Declarations and Essential Ethical Compliances

Authors' Contributions (in accordance with ICMJE criteria for authorship)

Contribution	Author 1	Author 2	Author 3	Author 4	Author 5
Conceived and designed the research or analysis	Yes	Yes	Yes	Yes	Yes
Collected the data	Yes	No	No	No	No
Contributed to data analysis & interpretation	Yes	No	No	No	No
Wrote the article/paper	Yes	No	No	No	No
Critical revision of the article/paper	Yes	Yes	Yes	Yes	Yes
Editing of the article/paper	Yes	Yes	Yes	Yes	Yes
Supervision	Yes	Yes	Yes	Yes	Yes
Project Administration	Yes	No	No	No	Yes
Funding Acquisition	Yes	No	No	No	Yes
Overall Contribution Proportion (%)	35	15	15	15	20

Funding
Research fund was available from UNEP/GEF through Alliance of Bioversity International-CIAT but no fund was available for writing and publication of this paper.

Research involving human bodies or organs or tissues (Helsinki Declaration)
The author(s) solemnly declare(s) that this research has not involved any human subject (body or organs) for experimentation. It was not a clinical research. The contexts of human population/participation were only indirectly covered through literature review. Therefore, an Ethical Clearance (from a Committee or Authority) or ethical obligation of Helsinki Declaration does not apply in cases of this study or written work.

Research involving animals (ARRIVE Checklist)
The author(s) solemnly declare(s) that this research has not involved any animal subject (body or organs) for experimentation. The research was not based on laboratory experiment involving any kind animal. The contexts of animals were only indirectly covered through literature review. Therefore, an Ethical Clearance (from a Committee or Authority) or ethical obligation of ARRIVE does not apply in cases of this study or written work.

Research on Indigenous Peoples and/or Traditional Knowledge
The author(s) solemnly declare(s) that this research has not involved any Indigenous Peoples as participants or respondents. The contexts of Indigenous Peoples or Indigenous Knowledge were only indirectly covered through literature review. Therefore, an Ethical Clearance (from a Committee or Authority) and Self-Declaration in this regard are appended.

Research involving Plants
The author(s) solemnly declare(s) that this research has involved the plants for experiment or field studies. Some contexts of plants are also indirectly covered through literature review. Thus, during this research the author(s) obeyed the principles of the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

Research Involving Local Community Participants (Non-Indigenous) or Children
The author(s) solemnly declare(s) that this research has directly involved local community participants or respondents belonging to non-Indigenous peoples. But, this study did not involve any child in any form directly. The contexts of different humans, people, populations, men/women/children and ethnic people are only indirectly covered through literature review. A sample copy of the Consent Form implying prior informed consent (PIC) of the respondents is appended.

(Optional) PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)
The author(s) has/have NOT complied with PRISMA standards. It is not relevant in case of this study or written work.

Competing Interests/Conflict of Interest
Author(s) has/have no competing financial, professional, or personal interests from other parties or in publishing this manuscript. There is no conflict of interest with the publisher or the editorial team or the reviewers.

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To see original copy of these declarations signed by Corresponding/First Author (on behalf of other co-authors too), please download associated zip folder [Declarations] from the published Abstract page accessible through and linked with the DOI: https://doi.org/10.33002/aa030101

Government of Nepal
NEPAL AGRICULTURAL RESEARCH COUNCIL
(Established by Government of Nepal under the Nepal Agricultural Research Council Act, 2048 B.S.)
NATIONAL AGRICULTURE GENETIC RESOURCES CENTER
(Genebank)

Ref. No.:

Dispatch No.:

Khumaltar, Lalitpur
Nepal

ETHICAL CLEARANCE CERTIFICATE

Research Involving Indigenous Peoples and Traditional Knowledge

Declaration by the Principal Investigator

I certify that the study titled: "Identifying Drought Tolerant Finger Millet Landraces for the Hills of Nepal", (ref: aa020202), **does not involve** collection of data or information on (an) Indigenous land, including reserve, settlement, and land governed under a self-government rule/agreement; the study **does not involve** any of the criteria for participation, including membership in an indigenous community, group of communities, or organization, including urban indigenous populations; the study **does not seek** inputs from participants (members of the indigenous community) regarding a community's cultural heritage, artifacts, traditional knowledge, biocultural or biological resources or unique characteristics/practices; and the study **does not involve** Aboriginal identity or membership in an indigenous community used or be used as a variable for the purpose of analysis. The present study is conducted on government research stations and **does not involve** any Indigenous Peoples or Communities. I hereby declare the same and confirm that all personnel associated with the present study have read this application and have agreed to comply with procedures described and any conditions imposed by the World Intellectual Property Organization (WIPO), Geneva, with regards to research on Indigenous Peoples and/or Traditional Knowledge.

Principal Investigator:

Date: 07-06-2023

Declaration by Head of the Organization/Research Committee

I have read this application and am satisfied that the study **does not involve** capturing and collection of data or information of the Indigenous Community's cultural heritage, artifacts, traditional knowledge, biocultural or biological resources or unique characteristics/practices. The study fully complies with the legislation and the general principles of the World Intellectual Property Organization (WIPO), Geneva.

Head of the Organization/Research Committee

Date: 7/6/23

Mailing Address: P.O.Box No. 3055, Kathmandu, Nepal
Phone: (+977-1) 5003125, 5003331, Fax: (+977-1) 5003331
E-mail: nagrc2010@yahoo.com, Website: http://www.genebank-narc.gov.np

SELF-DECLARATION FORM
Research on Indigenous Peoples and/or Traditional Knowledge

The nature and extent of community engagement should be determined jointly by the researcher and the relevant community or collective, taking into account the characteristics and protocols of the community and the nature of the research.

If your research involved/involves the Indigenous Peoples as participants or respondents, you should fill in and upload this Self-Declaration and/or Prior Informed Consent (PIC) from the Indigenous Peoples. [Please read carefully https://grassrootsjournals.org/credibility-compliance.php#Research-Ethics]

1. Conditions of the Research

1.1 Was or will the research (be) conducted on (an) Indigenous land, including reserve, settlement, and land governed under a self-government rule/agreement or?

No

1.2 Did/does any of the criteria for participation include membership in an Indigenous community, group of communities, or organization, including urban Indigenous populations?

No

1.3 Did/does the research seek inputs from participants (members of the Indigenous community) regarding a community's cultural heritage, artifacts, traditional knowledge, biocultural or biological resources or unique characteristics/practices?

No

1.4 Did/will Aboriginal identity or membership in an Indigenous community used or be used as a variable for the purposes of analysis?

No

2. Community Engagement

2.1 If you answered "Yes" to questions 1.1, 1.2, 1.3 or 1.4, have you initiated or do you intend to initiate an engagement process with the Indigenous collective, community or communities for this study?

Not Applicable

2.2 If you answered "Yes" to question 2.1, describe the process that you have followed or will follow with respect to community engagement. Include any documentation of

consultations (*i.e., formal research agreement, letter of approval, PIC, email communications, etc.*) and the role or position of those consulted, including their names if appropriate:

Not Applicable

3. No Community Consultation or Engagement

If you answered "No" to question 2.1, briefly describe why community engagement will not be sought and how you can conduct a study that respects Aboriginal/ Indigenous communities and participants in the absence of community engagement.

Not Applicable

Name of Principal Researcher: Krishna Hari Ghimire
Affiliation of Principal Researcher:

1. Senior Scientist (S-4), National Agriculture Genetic Resources Center, Nepal Agricultural Research Council, Nepal
2. PhD Student, Department of Genetics and Plant Breeding, Agriculture and Forestry University, Chitwan, Nepal

Signature:

Date: 7/6/2023

Declaration: Submitting this note by email to any journal published by The Grassroots Institute is your confirmation that the information declared above is correct and devoid of any manipulation.

INFORMATION AND CONSENT FORM FROM RESPONDENTS
(Non-Indigenous or Indigenous Respondents)
This form was translated into local language for the respondents

Title of the Research: Identifying Drought Tolerant Finger Millet Landraces for the Hills of Nepal

Principal Researcher: Krishna Hari Ghimire
National Agriculture Genetic Resources Center (Genebank)
Nepal Agricultural Research Council (NARC)

Research Supervisor: Self

A) INFORMATION TO PARTICIPANTS

1. Objectives of the research
To evaluate native finger millet landraces under drought stress condition, identify promising landraces and enhanced their utilization in developing high yielding varieties for the hills of Nepal.

2. Participation in research
The researcher will ask you several pertinent questions. This interview will be recorded in written form and should last about 50-60 minutes. The location and timing of the interview will be determined by you, depending on your availability and convenience.

3. Risks and disadvantages
There is no particular risk involved in this project. You may, however, refuse to answer any question at any time or even terminate the interview.

4. Advantanges and benefits
You will receive intangible benefits even if you refuse to answer some questions or decide to terminate the interview.

5. Confidentiality
Personal information you give us will be kept confidential. No information identifying you in any way will be published. In addition, each participant in the research will be assigned a code and only the researcher will know your identity.

6. Right of withdrawal
Your participation in this project is entirely voluntary and you can at any time withdraw from the research on simple verbal notice and without having to justify your decision, without consequence to you. If you decide to opt out of the research, please contact the researcher at the telephone number or email listed below. At your request, all information concerning you can also be destroyed. However, after the outbreak of the publishing process, it is impossible to destroy the analyses and results on the data collected.

B) CONSENT

Declaration of the participant

- ⇨ I understand that I can take some time to think before agreeing or not to participate in the research.
- ⇨ I can ask the research team questions and ask for satisfactory answers.
- ⇨ I understand that by participating in this research project, I do not relinquish any of my rights, including my right to terminate the interview at any time.
- ⇨ I have read this information and consent form and agree to participate in the research project.
- ⇨ I agree that the interviews be recorded in written form by the researcher: Yes ( ) No ( )

Signature of the participant : _____ Date : _____

Surname : _____ First name : _____

Researcher engagement

I explained to the participant the conditions for participation in the research project. I answered to the best of my knowledge the questions asked and I made sure of the participant's understanding. I, along with the research team, agree to abide by what was agreed to in this information and consent form.

Signature of the researcher: Date : 07-06-2023

Surname: Ghimire First name: Krishna Middle name: Hari

- ⇨ Should you have any questions regarding this study, or to withdraw from the research, please contact to Krishna Hari Ghimire by e-mail ghimirekh@gmail.com
- ⇨ If you have any concerns about your rights or about the responsibilities of researchers concerning your participation in this project, you can contact to National Agriculture Genetic Resources Center, Nepal Agricultural Research Council by email joshibalak@yahoo.com