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Assessment of Proso millet Genotypes against Blast Caused by *Pyricularia grisea* (Cooke) Sacc. under Field Condition at Mid Hill Region of Nepal

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ABSTRACT

A total of 14 proso millet genotypes, including the farmers' variety, which was the most commonly cultivated landrace in the Karnali region and most susceptible to blast disease as a susceptible check, were evaluated for resistance to leaf blast at the Hill Crops Research Program, Kabre, Dolakha, Nepal during the summer seasons of 2020 and 2021. The experiment was conducted under natural epiphytotic conditions. The experiment revealed that none of the tested genotypes were found immune or highly resistant. Most of the genotypes showed moderately susceptible and susceptible reactions to the leaf blast disease for both experimental years. However, genotypes C04654, Humla-239, and C04651 were found to have lower blast severity and produce higher grain yields. Genotype C04654 was found as resistant with 34.6% disease severity and high yielding (2.6 t/ha), followed by Humla-239 and C04651 as moderately susceptible with 44.8 and 45.9% disease severity resulting grain yields of 2.5 and 2.4 t/ha, respectively. The higher disease severity (68.1%) with lower grain yield (1.4 t/ha) was recorded in the farmers' variety (susceptible check). The genotypes reported with lower blast severity and higher grain yield could be the source for the release of a leaf blast resistant and high-yielding proso millet variety in the mid-hill region of Nepal.

Keywords: blast, proso millet, *Pyricularia grisea*, resistant, susceptible.

सारांश

पातमा लाग्ने मरुवा रोग प्रतिरोधी साथै उच्च उत्पादन दिने जातको पहिचान र विकास हेतु १४ वटा चिनोका जातहरु जसमा किसानले खेती गर्ने जातलाई संक्रमित चेक जातको रुपमा सन् २०२० र २०२१ को बर्षे सिजनमा पहाडी बाली अनुसन्धान कार्यक्रम, काब्रे, दोलखाको अनुसन्धान ब्लकमा, छनोट नर्सरीमा लगाई परिक्षणहरु संचालन गरिएको थियो । परिक्षणमा सम्मिलित कुनै पनि जातहरु रोग मुक्त साथै उच्च प्रतिरोधी भने पाइएन । धेरै जसो जातहरु पातमा लाग्ने मरुवा रोगसंग मध्यम संक्रमित तथा संक्रमित भएको पाइयो । तर पनि केही जातहरु जस्तै सी.०४६५४, हुम्ला-२३९ र सी.०४६५१ मा पातमा लाग्ने मरुवा रोगको प्रकोप कम र उत्पादन पनि बढी भएको पाइयो । सी.०४६५४ मा सबैभन्दा कम रोगको प्रकोप प्रतिशत (३४.६%) र उच्च उत्पादन (२.६ टन/हेक्टर), त्यसैगरी हुम्ला-२३९ र सी.०४६५१ मा रोगको प्रकोप प्रतिशत क्रमशः ३४.६% र ४५.९% साथै उत्पादन क्रमशः २.५ टन/हेक्टर र २.४ टन/हेक्टर भएको पाइयो । किसानले खेती गर्ने जात जो संक्रमित चेक जातको रुपमा लगाईएको थियो, उक्त जातमा सबैभन्दा बढी रोगको प्रकोप (६८.१%) र कम उत्पादन (१.४ टन/हेक्टर) भएको पाइयो । परिक्षणहरुको नतिजाबाट प्राप्त यी जातहरु नेपालको मध्य पहाडी क्षेत्रको लागि चिनोको पातमा लाग्ने मरुवा रोग प्रतिरोधी साथै उच्च उत्पादन दिने जातको विकास गर्न स्रोतको रुपमा उपयोगी सिद्ध हुनेछन् ।

INTRODUCTION

Millet is a small-seeded annual hardy cereal crop grown in the tropical and subtropical areas of the world. Among millet group, proso millet (*Panicum miliaceum* L.), also known as Chino in Nepal, is a tetraploid (2n=4x=36) species in the Poaceae family with great potential to combat food insecurity and is regarded as a future climate smart crop (Ghimire et al 2018). It is regarded as "Future Smart Food" due to its potential for food and nutritional security for a growing population and climate resilience capability (Gauchan et al 2020). Proso millet is high in protein (12.5%), fat (1.1%), carbohydrate (70.4%), fiber (2.2%), and minerals (1.9%) such as iron, zinc, copper, and manganese (Saud 2010, Saha et al 2016), all of which have antipyletic, antioxidant, and antibacterial properties (Kumar et al 2016). In Nepal, it is the second most important crop after finger millet among millet crops. The major districts producing proso millet are Mugu, Dolpa, Humla, Jumla, Kalikot, Bajura, and Jajarkot of the Karnali region (Shrestha et al 2020), with an average area of 2000 ha and a productivity of 0.81 t/ha (Ghimire et al 2018).

Although proso millet is adapted to a wide range of soils and has a unique climate resilience ability, biotic stress can cause heavy loss, damaging the entire crop (Sathiyabama and Manikandan 2016). Among various biotic stresses that affect proso millet, the incidence of blast caused by *Pyricularia grisea* (Cooke) Sacc [teleomorph: *Magnaporthe grisea* (Hebert) Barr.] is the most important disease (Gauchan et al 2020). Blast incidence is evident in all growth stages, producing elliptical leaf spots or whitish centers with reddish brown margins that infect the foliage, neck, and head of the crop, causing yield and biomass reduction in Nepal (Manandhar et al 2016). Prajapati et al (2013) reported significant losses in grain yield and fodder yield of 35.78 and 43.72%, respectively, with an average yield loss of around 28.36% (Nagaraja and Mantur 2007), with severe cases reporting up to 100% yield loss (HCRP 2020). Lower temperatures and higher humidity are ideal for disease progression, leading to epidemics of leaf, neck, and head blast (Manandhar et al 2016). Although blast can be efficiently controlled by using chemical fungicide (Prajapati et al 2013), it possesses an environmental hazard and is not economical, especially for underutilized and neglected crop species (Subedi et al 2020). Hence, sustainable management of blast can be obtained through host-plant resistance, which means utilization of the plant's own defense mechanism in management of pest and diseases, particularly the rapid localized cell death, also known as hypersensitive response (Bhatta et al 2017). So, this study was designed and conducted at the Hill Crops Research Program (HCRP), Kabre, Nepal to analyze and evaluate the level of resistance of different proso millet genotypes against blast disease and to identify superior genotypes for their yield-attributing characteristics, leaf blast resistance, and phenotypic characteristics that could be utilized in future breeding programs.

MATERIALS AND METHODS

An experiment was organized with fourteen proso-millet genotypes including a farmers' variety as a susceptible check following a randomized complete block design with two replications during the summer seasons of 2020 and 2021 under natural infestation at the field conditions of the Hill Crops Research Program (HCRP) Kabre, Dolakha, Nepal. The sources of all fourteen genotypes (C04651, C04645, C04656, C03149, Humla-237, Humla-239, Humla-312, Humla-383, Humla-488, Humla-530, Humla-653, Humla-725, and Farmers' variety) were HCRP, Kabre, and Dolakha. All genotypes were landraces, collected from the Karnali region (Humla and Jumla) and maintained in the breeding program of HCRP, Kabre, Dolakha. Farmers' variety was the most commonly cultivated landrace in the Karnali region and was more susceptible to blast disease and considered as susceptible to check. The geographic coordinates of the research field are 27 38' N latitude, 86 80' E longitude, and 1740 masl altitude. The soil is slightly acidic (pH 4.5-6.2), light-textured, and sandy loam. The average total annual rainfall was 2350 mm, with a distinct monsoon period (>80% of annual rainfall) from mid-June to mid-September. The unit plot size was 2 m × 2 m with a 25 cm row to row spacing and continuous plant to plant spacing was maintained. The net harvested plot was 4 square meters. The experiment was planted in the first week of May for both consecutive years. The recommended dose of fertilizer was 50:30:20 $N:P_2O_5:K_2O$ kg/ha and farm yard manure (FYM) was 8 t/ha with a seed rate of 10 kg/ha (HCRP 2019). Most of the cultural practices were followed as recommended (HCRP 2019).

Data on leaf blast disease severity was recorded from 10 randomly tagged plants/plot on the basis of a 1-9 scoring scale as described in finger millet (Kiranbabu et al 2013) during the grain formation stage. Yield and yield-attributing traits like days to heading, days to maturity, plant height (cm), leaf length (cm), leaf width (cm), panicle length (cm), node/tiller, grain yield (t/ha) and thousand grain weight (g) were recorded. The percent disease index (PDI) is computed on the basis of recorded data according to the formula (Wheeler 1969).

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of individual ratings}}{\text{No. of plants examined} \times \text{Maximum disease scale}} \times 100$$

All data was analyzed statistically using Microsoft Excel 2013 and GENSTAT 18th edition computer package programs. The relationship between the disease index and grain yield was also calculated.

RESULTS

Proso millet genotypes varied significantly (P≤0.05) for days to heading (DTH), days to maturity (DTM), plant height, leaf length, leaf width, node per tiller, panicle length, grain yield per spike, grain yield, thousand grain weight and leaf blast severity during FY 2020/21 (**Table** 1). The days to heading (52-60) ranged with the mean average of 56 ± 0.44, days to maturity (95-100) with the mean average of 98 ± 0.32, plant height (80.4-121.4 cm) with the mean average of 109 ± 1.92 cm, leaf length (36-57.6 cm) with the mean average of 47 ± 0.82 cm, leaf width (1.7-2.5 cm) with the mean average of 2.2 ± 0.04 cm, nodes per tiller (5-7.5) with the mean average of 5.9 ± 0.11, panicle length (36.2-44.8 cm) with the mean average of 40 ± 0.47 cm, grain yield per spike (6.4-11.5 g) with the mean average of 8.3 ± 0.24 g, grain yield (0.73-2.62 t/ha) with the mean average of 1.9 ± 0.08 t/ha, thousand grain weight (6-8 g) with the mean average of 7 ± 0.16 g and leaf blast severity (34.6-69.5%) with the mean average of 56.1±1.68% (**Table** 1).

Table 1. Statistical parameters of yield components, yield and leaf blast disease severity of proso millet genotypes evaluated at Kabre, Dolakha, Nepal during 2020/21

Parameters	Mean ± SEm	Range	P- value	LSD (0.05)	CV, (%)
Days to heading (DTH)	†56 ± 0.44	52-60	<.001	1.57	1.3
Days to maturity (DTM)	98 ± 0.32	95-100	<.001	0.94	0.4
Plant height (cm)	109 ± 1.92	80.4-121.4	<.001	2.07	0.9
Leaf length (cm)	47 ± 0.82	36-57.6	<.001	1.98	2.0
Leaf width (cm)	2.2 ± 0.04	1.7-2.5	0.029	0.31	6.6
Nodes/tiller	5.9 ± 0.11	5-7.5	0.002	0.69	5.4
Panicle length (cm)	40 ± 0.47	36.2-44.8	<.001	1.69	2.0
Grain yield/spike (g)	8.3 ± 0.24	6.4-11.5	<.001	0.74	4.2
GY (t/ha)	1.9 ± 0.08	0.73-2.62	<.001	0.16	3.9
TGW (g)	7 ± 0.16	6-8.0	<.001	0.78	5.2
Leaf blast PDI %	56.1 ± 1.68	34.6-69.5	<.001	1.32	1.1

†Means of 2 replications, SEm- standard error mean, GY- grain yield, TGW- thousand grain weight, PDI-percent disease index, %- percentage, cm-centimeter, t/ha- ton per hectare, g- gram

During 2021/22 also similar trends were reported and data revealed that statistically highly significant differences for the parameters days to heading (DTH), days to maturity (DTM), plant height, leaf length, leaf width, node per tiller, panicle length, grain yield per spike, grain yield, thousand grain weight and leaf blast severity among the tested proso millet genotypes (**Table** 2).The days to heading

(55-64) ranged with the mean average of 58 ± 0.41, days to maturity (94-98) with the mean average of 96 ± 0.21, plant height (110.2-131.2 cm) with the mean average of 121.3 ± 1.12 cm, leaf length (22.8-32.8 cm) with the mean average of 27.3 ± 0.38 cm, leaf width (2.2-3.0 cm) with the mean average of 2.6 ± 0.04 cm, nodes per tiller (5.6-7.6) with the mean average of 6.4 ± 0.10, panicle length (25.8-30.2 cm) with the mean average of 28 ± 0.23 cm, grain yield per spike (2.8-7.3 g) with the mean average of 5 ± 0.22 g, grain yield (1.39-2.92 t/ha) with the mean average of 2.3 ± 0.07 t/ha, thousand grain weight (5.5-7 g) with the mean average of 6 ± 0.16 g and leaf blast severity (32.5-67.8%) with the mean average of 54.7±1.70% (**Table** 2).

The combined mean performance of proso millet genotypes for the leaf blast disease severity, yield and yield components during 2020-2021 shown in **Table** 3. Statistically highly significant differences were observed for the parameters days to heading (DTH), days to maturity (DTM), plant height, leaf length, leaf width, node per tiller, panicle length, grain yield per spike, grain yield, thousand grain weight and leaf blast severity among the tested proso millet genotypes in combined analysis for two consecutive years. The days to heading (55-61) ranged with the mean average of 57, days to maturity (95-99) with the mean average of 97, plant height (103.1-123.2 cm) with the mean average of 115.1 cm, leaf length (32.1-42 cm) with the mean average of 37.1 cm, leaf width (2.1-2.6 cm) with the mean average of 2.4 cm, nodes per tiller (6-7) with the mean average of 6, panicle length (32-36.2 cm) with the mean average of 33.9 cm, grain yield per spike (5.6-8.1 g) with the mean average of 6.6 g, grain yield (1.4-2.6 t/ha) with the mean average of 2.1 t/ha, thousand grain weight (6.1-7.4 g) with the mean average of 6.7 g and leaf blast severity (34.6-68.1%) with the mean average of 55.4 % (**Table** 3). The lower percent disease index and higher yield were recorded in genotypes C04654 (PDI-34.6% and GY-2.6 t/ha), Humla-239 (PDI-44.8% and GY-2.5 t/ha) and C04651 (PDI-45.9% and GY-2.4 t/ha), respectively (**Table** 3).

Table2. Statistical parameters of yield components, yield and leaf blast disease severity of proso millet genotypes evaluated at Kabre, Dolakha, Nepal during 2021/22

Parameters	Mean ± SEm	Range	P- value	LSD (0.05)	CV, (%)
Days to heading (DTH)	†58 ± 0.41	55-64	0.02	3.20	2.60
Days to maturity (DTM)	96 ± 0.21	94-98	0.02	1.64	0.80
Plant height (cm)	121.3 ± 1.12	110.2-131.2	<.001	5.92	2.30
Leaf length (cm)	27.3 ± 0.38	22.8-32.8	0.002	2.43	4.10
Leaf width (cm)	2.6 ± 0.04	2.2-3.0	0.01	0.31	5.60
Nodes/tiller	6.4 ± 0.10	5.6-7.6	<.001	0.33	2.40
Panicle length (cm)	28 ± 0.23	25.8-30.2	0.281	2.45	4.00
Grain yield/spike (g)	5 ± 0.22	2.8-7.3	<.001	0.59	5.5
GY (t/ha)	2.3± 0.07	1.39-2.92	<.001	0.05	0.9
TGW (g)	6 ± 0.07	5.5-7.0	0.339	0.71	5.10
Leaf blast PDI %	54.7 ± 1.70	32.5-67.8	<.001	1.49	1.3

†Means of 2 replications, SEm- standard error mean, GY- grain yield, TGW- thousand grain weight, PDI-percent disease index, %- percentage, cm-centimeter, t/ha- ton per hectare, g- gram

Table 3. Combined mean performance of prosomillet genotypes to the yield, yield components and leaf blast disease severity during 2020-2022 at Kabre, Dolakha, Nepal

Genotypes	DTH	DTM	PH (cm)	LL (cm)	LW (cm)	Node/ tiller	PL (cm)	TGW (g)	GY/ S (g)	GY (t/ha)	Blast PDI %
C04651	†59	98	112.5	36.6	2.6	6	34.9	6.4	6.6	2.4	45.9
C04645	57	97	111.1	36.9	2.5	6	32.6	7.0	6.0	2.3	52.8
C04654	55	96	121.7	39.8	2.2	6	33.0	7.4	6.8	2.6	34.6
C04656	61	98	120.6	42.0	2.3	7	36.2	6.4	7.5	2.2	55.4
C03149	56	96	114.8	37.7	2.5	6	33.0	6.9	7.2	2.1	57.2
Humla-237	55	96	119.5	39.0	2.6	6	33.1	7.2	5.6	1.7	64.6
Humla-239	55	98	119.1	36.0	2.3	6	33.0	6.9	5.6	2.5	44.8
Humla-312	60	98	109.5	38.6	2.3	6	35.2	7.0	6.2	1.6	64.9
Humla-383	57	99	106.1	35.7	2.1	6	33.2	6.3	5.6	2.2	55.3
Humla-488	57	96	119.8	36.6	2.4	6	34.9	6.1	7.8	2.2	53.8
Humla-530	55	95	114.9	35.8	2.3	6	34.6	7.1	6.0	2.0	57.4
Humla-653	58	97	116.3	34.9	2.6	6	34.2	6.9	7.9	1.8	63.8
Humla-725	56	97	123.2	32.1	2.3	7	32.0	6.1	5.7	2.1	56.8
Farmers' variety (SC)	58	98	103.1	38.1	2.5	6	35.5	6.8	8.1	1.4	68.1
Grand mean	57	97	115.1	37.1	2.4	6	33.9	6.7	6.6	2.1	55.4
Min	55	95	103.1	32.1	2.1	6	32.0	6.1	5.6	1.4	34.6
Max	61	99	123.2	42.0	2.6	7	36.2	7.4	8.1	2.6	68.1
Genotype (G)	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Year (Y)	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
Genotype × Year	0.042	<.001	<.001	<.001	0.104	<.001	<.001	<.001	<.001	<.001	1.00
$LSD_{0.05}$ (G)	1.67	0.88	3.27	1.50	0.21	0.37	1.39	0.53	0.44	0.08	1.73
$LSD_{0.05}$ (Y)	0.63	0.33	1.24	0.57	0.08	0.14	0.53	0.20	0.17	0.03	0.66
$LSD_{0.05}$ (G×Y)	2.37	1.25	4.62	2.12	0.29	0.53	1.97	0.75	0.63	0.11	2.45
CV,%	2.00	0.60	2.00	2.80	6.00	4.20	2.80	5.40	4.60	2.60	2.20

†Means of 2 replications, DTH-days to heading, DTM-days to maturity, PH-plant height, LL-leaf length, LW-leaf width, PL-panicle length, TGW-thousand grain weight, S-spike, GY-grain yield, PDI-percent disease index, cm-centimeter, g-gram, t/ha-ton per hectare

Relationship between disease index (PDI) and grain yield

The best fit, with adjusted R^2 = 86%, showed a substantial linear negative association (r = -0.93) between grain yield and leaf blast disease severity (**Figure** 1). Consequently, as disease severity (PDI) increased, the yield dropped. The projected linear regression line has a decreasing slope as well, i.e y =-0.033 x+3.949, with a regression coefficient R^2 = 0.87, where "y" denoted predicted grain yield (t/ha) of proso millet genotypes and "x" stood for percent disease index of leaf blast of proso millet genotypes (**Figure** 1).

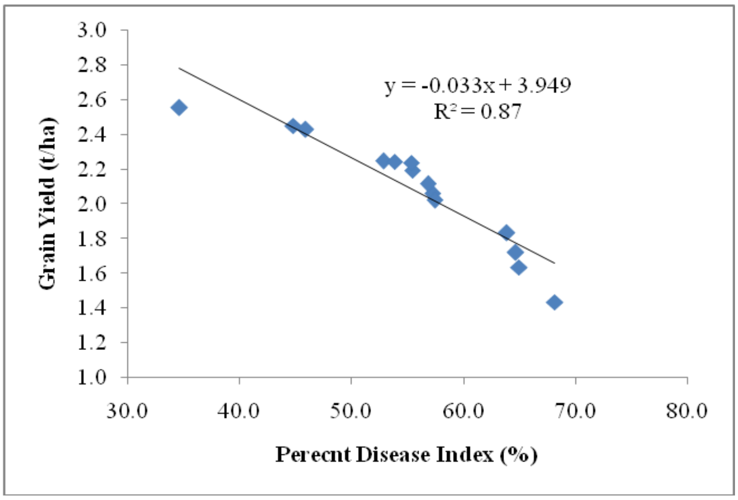

Figure 1: Relationship between grain yield (t/ha) and blast disease severity (PDI) of proso millet genotypes at HCRP, Kabre, Dolakha, Nepal during 2020-2021

Weather parameters during crop growing period (2020/21)

The climatic factors during the crop growing period (May to September) at HCRP, Kabre, Dolakha for both consecutive years 2020 and 2021 are shown in **Figure** 2. During 2020, the average monthly minimum temperature ranged from 15.9 °C in the month of May to 19.8 °C in August. Similarly, the average monthly maximum temperature ranged from 25 °C in May to 27.3 °C in September. The total rainfall during the crop growing period was 1676.5 mm, where higher rainfall (601.7 mm) was recorded in the month of July. The average monthly relative humidity ranged from 89.8% in May to 91.6% in July. A similar trend was followed during the crop growing period of 2021. The average monthly minimum temperature ranged from 16.7 °C in the month of May to 19.8 °C in the month of September. Similarly, the average monthly maximum temperature ranged from 25.1 °C in May to 27.7°C in September. The total rainfall during the crop growing period was 1645.8 mm, where higher rainfall (590.5 mm) was recorded in the month of July. The average monthly relative humidity ranged from 83% in June to 90.5% in September (**Figure** 2).

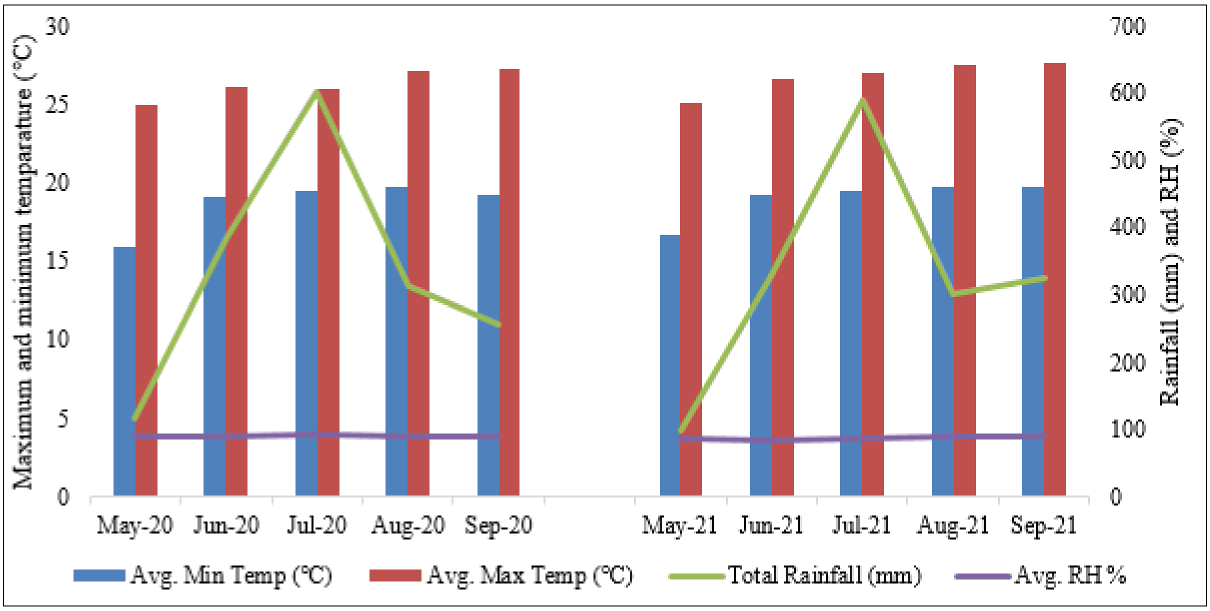

Figure 2: Weather parameters during crop growing period (May-September) at HCRP, Kabre, Dolakha, Nepal during year 2020 and 2021.

DISCUSSIONS

The incidence and severity of the disease are primarily regulated by environmental factors. The climatic evidence showed that the incidence and severity of blast disease at HCRP, Kabre, and Dolakha during the crop growing period were prominent, with temperatures ranging from 19–25 °C, higher rainfall (> 1600 mm), and high relative humidity (> 85%) for both consecutive years. Temperature and precipitation are the most important climatic factors for the onset of the disease cycle for blast (Bevitori and Ghini 2014). Low temperatures, high relative humidity (>80%), and higher rainfall were conducive to blast formation, particularly leaf and head blasts (Nagaraja et al 2010). Based on two years of results, proso millet genotypes C04654, Humla-239, and C04651 have been found to have lower leaf blast severity and higher grain yield. The finding of this experiment is in line with the result of HCRP (2019), which showed that proso millet genotypes C04651, C04654, Humla-239, and Humla-383 were high-yielding, stable, and blast-resistant. Similarly, Kandel et al (2020) also reported that C04656, C04654, Humla-383, and Humla-239 were found to be more stable, high-yielding, and adaptive genotypoes with lower blast severity across the hill regions of Nepal. Significant variation in blast reactions was observed in genotypes grown under different agro-geographical conditions (Ramakrishnan et al 2016). Moreover, Khadka et al (2013) observed significantly diverse virulence of isolates in the location from where isolates were collected, which indicates that the genetic makeup of the organism is regulated by geographical isolation and specificity. Sreenivasaprasad et al (2001) reported that blast isolates were more or less aggressive on all the millet varieties tested, and there was no distinct difference in compatibility among various fungus isolates and millet varieties, which indicates that there are no major genes involved for resistance in these interactions.

Plant Reactive Oxygen Species (ROS) are important molecules in plant defense mechanisms (Nanda et al 2010), and H_2O_2 is recognized to be a local signal that mediates the induction of plant defensive responses as well as the expression of PR (pathogen-related) genes (Sathiyabama and Manikandan 2016). They are signal molecules crucial for plant defense mechanisms (Anusuya and Sathiyabama 2015), which are involved in plant-pathogen interactions (Apel and Hirt 2004) as well as programmed cell death (Gechev and Hille 2005) and stress responses.

CONCLUSION

Genotypes C04654, Humla-239, and C04651 possessed moderate level of resistance to leaf blast and produced higher grain yields. Thus, these genotypes could be cultivated directly as blast-resistant and high-yielding in the mid-hill region of Nepal or may be utilized in breeding programs for developing higher-yielding varieties with blast resistance.

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