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World's first black-seeded high yielding mungbean [*Vigna radiata* (L.) Wilczek] varieties 'NIFA Sikaram-21 and NIFA Spinghar-21'

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Abstract
Globally, black-seeded mungbean is cultivated only in Kuram area of Khyber Pakhtunkhwa (KP) of Pakistan and adjacent areas of Afghanistan. The black-seeded mungbean land-race being cultivated in the area since unknown dates had poor genetic back-ground, and hence, low yield potential and susceptibility to diseases. In order to develop high yielding shiny black-seeded mungbean varieties for Kuram, a cross was attempted between a local Mungbean Yellow Mosaic Virus (MYMV) susceptible land-race collected from Kuram and later named as Kuram black mung (KBM) and an MYMV resistant black mottled-seeded mutant named as NIFA black mung (NBM) in the kharif season 2014 at the Nuclear Institute for Food & Agriculture (NIFA), Peshawar. The F_1 generation was planted in spring 2015. The F_2 to F_5 generations were raised in kharif 2016 – spring 2019. Single plants and lines based on shiny black seed coat color, short and medium plant stature, MYMV resistance and high grain yield were selected. Elite short and medium statured lines NBM-2-2-4-5 (NIFA Sikaram-21) and NBM-5-3-7 (NIFA Spinghar-21), respectively, were selected and tested in replicated yield trials along with black-seeded parents and green-seeded check varieties at NIFA and other locations in Kuram. On an average, NIFA Sikaram-21 produced seed yield of 1716 kg ha^{-1} and NIFA Spinghar-21 1724 kg ha^{-1} against KBM (880 kg ha^{-1}) with 94% increase over KBM. However, its yield was lower than those of the green-seeded checks as green-seeded mungbean is well adapted to the growing conditions. Shiny black seeds, bold seed size and high grain yield are the distinguishing characters of NIFA Sikaram-21 and NIFA Spinghar-21. Furthermore, these are the first-ever black-seeded mungbean approved varieties in the world. The Provincial Seed Council of the KP approved both varieties in its 40th meeting held on April 07, 2021.

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Introduction

Mungbean is an important kharif season's crop grown on marginal lands of Pakistan including the Khyber Pakhtunkhwa (KP) province. It was grown on an area of 0.172 million ha with a total production of 0.125 million tons (Agric. Statistics of Pak., 2020). In KP, mungbean was grown on an area of 0.006 million hectares with a total production of 0.002 million tons (Agric. Statistics of Pak., 2020). In KP, a major portion of the area under mungbean cultivation lies in the Kuram district meaning that there is a great potential for expanding mungbean area in KP by bringing more area under cultivation in Kuram.

Currently, mungbean being cultivated globally has green seed coat color as it is preferred by the consumers and has good market value, whereas no breeding work has so far been reported on mungbean having black seed coat color. The reason behind this is the consumer's preference for

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mungbean having green seed coat color globally. There is neither structural difference in black and green mungbean plants nor do they differ in nutritional compositions; the only difference is the color of seed coat. In Kuram district, the mungbean growers are accustomed to grow mungbean having black seed coat color, because of the high demand from consumers. Plant breeding also emphasizes on breeding crop plants based on consumers' preferences, so that a newly developed crop variety is readily adopted by the growers. The importance of crop breeding based on consumers' preferences has previously been reported by several researchers (Carlson, 2007; Kassie et al., 2017; Wale and Yalew, 2007; Poudel and Jhonsen, 2009; Asrat et al., 2010; Blazy et al., 2011; Smith and Fennessy, 2011). Development of high yielding black-seeded mungbean varieties adapted to the growing conditions of Kuram was therefore felt necessary. The local land race being cultivated there for centuries had low yield potential due to poor genetic background for seed yield. The Nuclear Institute for Food and Agriculture (NIFA), Peshawar started breeding work with a financial assistance from the Pakistan Science Foundation (PSF) on the improvement of black-seeded mungbean, particularly for Kuram. The efforts resulted in breeding of two high yielding and disease resistant black-seeded mungbean advanced lines NBM-2-2-4-5 and NBM-5-3-7. These advanced lines were further evaluated in mandatory replicated yield trials, which are pre-requisite for variety approval with the financial support of a Pulses PSDP project, PARC. As a result, two high yielding black-seeded mungbean varieties namely NIFA Sikaram-21 and NIFA Spinghar-21 were released for general cultivation in Kuram during 2021. These varieties would play a key role in enhancing overall production of mungbean in the country, in general, and KP, in particular.

The current manuscript describes the development process of these two black-seeded and high yielding mungbean varieties.

Materials and Methods

The seeds of the black-seeded mungbean land race grown in Kuram were collected from the local growers in spring 2013. The Kuram's land race had variation for dull and shiny black color. The seeds were separated on the basis of dull and shiny seed coat color and confirmed their breeding behavior by growing the seeds separately at NIFA, Peshawar. The true shiny black seeded rows were harvested and bulked, whereas the dull black-seeded rows were discarded as dull color was not preferred by the consumers. The Kuram shiny black-seeded land race was named as Kuram black mung (KBM) which was highly susceptible to MYMV in kharif season under NIFA's growing conditions apart from its very low yield potential. Another black mottled seeded mutant mungbean genotype developed at the Nuclear Institute for Agriculture Biology (NIAB) during 1996-99 and hereby named as NIFA black mung (NBM) was also used in this research work. KBM and NBM were hybridized as a single cross-combination in kharif 2014 according to Khattak et al. (1998) and F_1 generation was raised in spring 2015. All recombinant plants were harvested, threshed and bagged individually, and planted as plant-progeny-rows in F_2 generation in kharif 2016. Single plant selections were made based on more pods, short and medium plant stature, shiny black seed coat color, and resistance to MYMV. The selected single plants were space-planted in F_3 generation in kharif 2017 and single plants selections were again made based on the desired criteria. The F_4 generation was raised as plant-progeny-rows in kharif 2018 for generation advancement and confirmation of genetic behavior of the target traits. Single row/line selection was carried-out on the basis of traits mentioned above.

The segregating material was screened for MYMV resistance/susceptibility according to the procedure reported by Khan et al. (2007). To intensify MYMV inoculums from natural sources, a highly susceptible check "VC 1560D" was planted in the segregating populations. Individual plants were scored for MYMV percent infection four weeks after planting at the stage when all plants of the susceptible check were completely infected by MYMV.

True breeding lines selected for the desired traits were evaluated for yield and related traits in a replicated yield trial at NIFA and other locations in KP during 2019 and 2020. Each trial was laid-out in a Randomized Complete Block Design with each entry comprising four rows of four meter length with row-to-row and plant-to-plant spacing of 30 cm and 10 cm, respectively. Each plot of each entry was replicated three times. The experiment was sown under irrigated conditions and need-based irrigation was applied. Statistical analyses were done according to Steel and Torrie (1980). The lines were also screened for MYMV in a separate nursery at NIFA in the same seasons. The breeding history of NIFA Sikaram-21 (recombinant NBM-2-2-4-5) and NIFA Spinghar-21 (recombinant NBM-5-3-7) is given in **Table 1**.

Table 1. Breeding history of NIFA Sikaram-21 (NBM-2-2-4-5) and NIFA Spinghar-21 (NBM-5-3-7)

Season	F. Gen./Trial	Remarks
Kharif 2014	Hybridization	A cross between KBM and mutant NBM was attempted at NIFA, Peshawar. KBM was used as female parent
Spring 2015	F_1	F_1 generation of cross-combination 'KBM x NBM' along with parents was raised at NIFA. All recombinant plants were picked, threshed and bagged individually
Kharif 2016	F_2	F_2 along with parents was raised at NIFA as single-plant-progeny rows from plants harvested from F_1. The population was screened for MYMV by repeatedly planting VC1560D (a susceptible check) in the population. Single plant selections were made on the basis of more number of pods, shiny black seed coat color, short and medium plant stature, and resistance to MYMV.
Kharif 2017	F_3	Single plants selected from F_2 were planted at NIFA as plant-progeny-rows in F_3 generation for generation advancement and confirmation of breeding behavior of selections for traits of interest. Single plants with traits mentioned above were again selected based on visual observations in the field and post-harvest grading.
Kharif 2018	F_4	The population was raised to advance segregating generation and confirm the breeding behavior of selections for traits of interest at NIFA along with screening for MYMV resistance. Lines with true breeding behavior for traits of interest as mentioned above were selected based on visual observation in field and post-harvest grading.
Spring 2019	F_5	The selected high yielding, shiny black-seeded and MYMV resistant lines were planted at NIFA as line-progeny-rows for confirmation of the breeding behavior of the traits of interest. Better performing lines were selected for evaluation for yield and related traits in replicated yield trials at NIFA and other locations in KP.
Kharif 2019	Yield trials and screening against MYMV	The uniform and true breeding lines selected for shiny black seed coat color, high yield and MYMV resistance were evaluated for yield and related traits in replicated yield trial at NIFA and on different locations in Kuram. The lines were also screened for MYMV in a separate nursery at NIFA.
Spring & kharif 2020	Yield trials and screening against MYMV	The selected true breeding lines were again evaluated for yield and related traits in replicated yield trial at NIFA and on different locations in Kuram. The lines were also again screened for MYMV in a separate nursery at NIFA. Elite recombinant lines NBM-2-2-4-5 (short statured) and NBM-5-3-7 (medium statured) with shiny black seed coat color, high yield potential and MYMV resistance were evaluated for distinctness, uniformity and stability (DUS) studies.

Results and Discussion

The growth and yield performance of NIFA Sikaram-21 (NBM-2-2-4-5) and NIFA Spinghar-21 (NBM-5-3-7) in various replicated yield trials conducted at NIFA and other locations in KP is presented in **Tables 2 to 7**. In replicated yield trials conducted at NIFA in kharif 2019 and spring 2020, the recombinant lines NBM-5-3-7 (NIFA Spinghar-21) and NBM-2-2-4-5 (NIFA Sikaram-21) produced statistically significant higher ($P \leq 0.05$) seed yield of 1794 and 1734 kg ha^{-1} (kharif 2019) and 1715 and 1745 kg ha^{-1} (spring 2020), respectively, compared with the black-seeded parents NBM (1655 and 1542 kg ha^{-1}, respectively) and KBM (903 kg ha^{-1}) (**Table 2 & 3**). The parent KBM did not thrive well in kharif due to its high susceptibility to MYMV which prevails in the season due to occurrence of white fly (vector of MYMV). However, their yields were statistically lower than those of the green-seeded check varieties NIFA Mung-19 (1806 and 1756 kg ha^{-1}, respectively) and Ramzan (1790 and 1694 kg ha^{-1}) (**Table 2 & 3**). Similarly, in the replicated yield trial conducted at NIFA, Peshawar, in kharif 2020, NBM-5-3-7 (NIFA Spinghar-21) and NBM-2-2-4-5 (NIFA Sikaram-21) produced statistically significant ($P \leq 0.05$) higher seed yields of 1815 and 1775 kg ha^{-1}, respectively, compared with the parent NBM (1669 kg ha^{-1}), whereas the parent KBM did not thrive well in the season due to its susceptibility to MYMV (**Table 4**). Again, the yields of NIFA Spinghar-21 and NIFA Sikaram-21 were lower than those of the green-seeded check varieties Ramzan and NIFA Mung-19 with individual seed yield of 1789 and 1826 kg ha^{-1}, respectively (**Table 4**). In adaptability yield trials planted on farmers' fields at five locations in Kuram in kharif 2019, NBM-2-2-4-5 (NIFA Sikaram-21) and NBM-5-3-7 (NIFA Spinghar-21) produced significantly ($P \leq 0.05$) higher average seed yields of 1622 and 1613 kg ha^{-1}, respectively, compared with those of parents KBM (852 kg ha^{-1}) and

NBM (1489 kg ha^{-1}) (**Table 5**). However, the green-seeded check variety NIFA Mung-19 surpassed both lines with an average seed yield of 1630 kg ha^{-1} (**Table 5**). Similarly, in the adaptability yield trial conducted on the same locations in Kuram in kharif 2020, both lines out-yielded both parents with statistically significant ($P \leq 0.05$) average higher seed yields of 1703 and 1684 kg ha^{-1} against average seed yields of parents KBM (804 kg ha^{-1}) and NBM (1553 kg ha^{-1}), however, their yields were lower than the average seed yield of the green-seeded check variety NIFA Mung-19 with a value of 1737 kg ha^{-1} (**Table 6**). As a mandatory requirement for new varieties, Distinctness, Uniformity and Stability (DUS) study of these two candidate varieties was carried-out by the Federal Seed Certification and Registration Department (FSC & RD), KP region in spring and kharif 2020 at NIFA, Peshawar. New varieties of different crops with a high yield potential help enhance per unit yield as well as overall production of those crops (Ahmad et al., 2007; Wasin, 2007; Zulfiqar and Hussain, 2014; Joshi et al., 2017), whereas high economic yield potential is a trait of high interest to the growers (Hossain, 2012; Walker et al., 2015). The high yield potential of NIFA Sikaram-21 and NIFA Spinghar-21 (**Tables 2 to 6**) against the average yield potential of the black-seeded parents KBM and NBM will play a key role in enhancing overall mungbean production in the country, in general, and the KP, in particular, as well as expanding national mungbean area.

For a new variety to be successful, its yield potential should surpass the already existing commercial varieties of that crop under cultivation. In our case, both lines produced percent average increase of 40% in seed yield over those of parents KBM and NBM in Kuram with even higher individual values over the parents in Kuram, meaning that both varieties have a promising potential in Kuram for which these varieties have been developed. However, their yields were lower than those of the green-seeded check varieties at all locations in all seasons. The underlying reason for this could be the extensive breeding work on green-seeded mungbean and its adaptability world-wide except at Kuram of the KP, where only black-seeded mungbean is cultivated on a large area and the inhabitants prefer to consume it in huge quantities. With the passage of time and continuous breeding work, the black-seeded mungbean yield will definitely surpass the yield of green-seeded mungbean varieties.

Table 2. Performance of NIFA Sikaram-21 (NBM-2-2-4-5) and NIFA Spinghar-21 (NBM-5-3-7) in replicated yield trial conducted at NIFA, Peshawar in kharif 2019

S.No.	Entry	Parentage/Pedigree	DF (50%)	DM (90%)	PH (cm)	1000-SW (g)	SY (kg ha^{-1})
1	NBM-2-2-4-2	KMB × NBM	41	85	50	49	1404
2	NBM-2-2-4-5	-do-	40	81	51	49	1734
3	NBM-2-14-4-3	-do-	40	84	63	46	1351
4	NBM-2-15-7-1	-do-	39	86	63	48	1328
5	NBM-5-3-7	-do-	41	84	69	50	1794
6	NBM-5-3-9	-do-	44	85	64	52	1502
7	KBM	Parent (BS)	Did not thrive due to MYMV in the season				
8	NBM	-do-	40	84	74	48	1655
9	Ramzan	Std. check (GS)	44	77	53	49	1790
10	NIFA mung-19	-do-	41	81	65	49	1806
CV (%)			1.34	0.98	2.98	1.79	7.23
LSD (5%)			0.97	1.42	3.04	1.46	75.12

DF: Days to flowering, DM: Days to maturity, PH: Plant height, SW: Seed weight, BS: Black seeded, GS: Green seeded, SY: Seed yield

Table 3. Performance of NIFA Sikaram-21 (NBM-2-2-4-5) and NIFA Spinghar-21 (NBM-5-3-7) in replicated yield trial conducted at NIFA, Peshawar in spring 2020

S.No.	Entry	Parentage/Pedigree	DF (50%)	DM (90%)	PH (cm)	1000-SW (g)	SY (kg ha^{-1})
1	NBM-2-2-4-2	KMB × NBM	42	82	49	47	1125
2	NBM-2-2-4-5	-do-	39	85	50	50	1745
3	NBM-2-14-4-3	-do-	41	88	61	41	1014
4	NBM-2-15-7-1	-do-	39	86	61	45	1167
5	NBM-5-3-7	-do-	40	85	66	50	1715
6	NBM-5-3-9	-do-	39	86	60	49	1387
7	KBM	Parent (BS)	42	89	48	38	903
8	NBM	-do-	40	87	71	49	1542
9	Ramzan	Std. check (GS)	39	83	53	48	1694
10	NIFA mung-19	-do-	41	82	63	49	1756
CV (%)			1.12	1.63	3.03	1.84	5.88
LSD (5%)			0.77	2.38	3.00	1.47	141.32

DF: Days to flowering, DM: Days to maturity, PH: Plant height, SW: Seed weight, BS: Black seeded, GS: Green seeded, SY: Seed yield

Table 4. Performance of NIFA Sikaram-21 (NBM-2-2-4-5) and NIFA Spinghar-21 (NBM-5-3-7) in replicated yield trial conducted at NIFA, Peshawar in kharif 2020

S.No.	Entry	Parentage /Pedigree	DM (90%)	PH (cm)	1000-SW (g)	SY(kg ha^{-1})
1	NBM-2-2-4-2	KMB × NBM	83	52	49	1415
2	NBM-2-2-4-5	-do-	87	52	49	1775
3	NBM-2-14-4-3	-do-	88	67	43	1104
4	NBM-2-15-7-1	-do-	87	69	47	1226
5	NBM-5-3-7	-do-	86	73	50	1815
6	NBM-5-3-9	-do-	85	70	49	1611
7	KBM	Parent (BS)	Did not thrive due to MYMV in the season			
8	NBM	-do-	87	76	49	1669
9	Ramzan	Std. check (GS)	86	55	47	1789
10	NIFA mung-19	-do-	86	68	49	1826
CV (%)			1.22	3.64	1.36	6.51
LSD (5%)			1.81	3.86	1.08	91.79

DM: Days to maturity, PH: Plant height, SW: Seed weight, BS: Black seeded, GS: Green seeded, Seed yield

Table 5. Performance of NIFA Sikaram-21 (NBM-2-2-4-5) and NIFA Spinghar-21 (NBM-5-3-7) in adaptability yield trials planted at different locations on farmers' fields at Kuram in kharif 2019

Entry	PP	DM	PH	SW	Seed yield (kg ha^{-1})						PAI
					Malana	Boshara	Shanai	Shublan	Zeran	Av.	
NBM-2-2-4-5	KN	76	57	49	1750	1717	1626	1411	1613	1622	38
NBM-2-14-4-3	-do-	76	72	40	1438	1124	951	1180	1423	1223	-
NBM-2-15-7-1	-do-	74	71	48	1266	1284	1110	1131	1076	1173	-
NBM-5-3-7	-do-	75	75	49	1723	1691	1383	1419	1594	1613	37
KBM	BS	72	58	39	950	961	796	715	836	852	-
NBM	-do-	77	77	49	1634	1669	1640	1385	1433	1489	-
NIFA Mung-19	GS	75	71	49	1814	1797	1468	1449	1623	1630	-
CV (%)		1.38	2.80	1.73	2.50	6.93	8.46	5.09	6.60	6.87	-
LSD (5%)		1.13	3.04	2.30	3.02	76.18	88.39	74.15	81.91	80.83	

DM: Days to 90% maturity, PH: Plant Height (cm), SW: 1000 Seed weight (g); KN: KMB×NBM, BS: Black seeded (Parent), GS: Green seeded (Std. check), P1: Parent 1 (KBM), P2: Parent 2 (NBM); PAI: Percent average increase in yield over parents

Table 6. Performance of NIFA Sikaram-21 (NBM-2-2-4-5) and NIFA Spinghar-21 (NBM-5-3-7) in adaptability yield trials planted at different locations on farmers' fields at Kuram in kharif 2020

Entry	PP	DM	PH	SW	Seed yield (kg ha^{-1})						PAI
					Malana	Boshara	Shanai	Shublan	Zeran	Av.	
NBM-2-2-4-5	KN	75	58	49	1787	1626	1774	1590	1737	1703	44
NBM-2-14-4-3	-do-	74	70	42	1126	1216	1503	1147	1438	1328	-
NBM-2-15-7-1	-do-	73	72	46	1513	1015	1328	1003	1385	1249	-
NBM-5-3-7	-do-	74	73	49	1785	1605	1744	1556	1723	1684	42
KBM	BS	72	56	40	533	714	867	929	978	804	-
NBM	-do-	76	78	47	1634	1440	1648	1405	1641	1553	-
NFM-2019	GS	74	69	49	1835	1699	1779	1605	1765	1737	-
CV (%)		1.73	2.50	1.15	7.62	5.62	5.28	6.40	8.01	6.59	-
LSD (5%)		2.27	3.02	1.12	97.46	108.41	114.59	99.32	154.53	114.86	-

DM: Days to 90% maturity, PH: Plant Height (cm), SW: 1000 Seed weight (g); KN: KMB×NBM, BS: Black seeded (Parent), GS: Green seeded (Std. check), P1: Parent 1 (KBM), P2: Parent 2 (NBM); PAI: Percent average increase in yield over parents

Table 7: Reaction of NIFA Sikaram-21 (NBM-2-2-4-5) and NIFA Spinghar-21 (NBM-5-3-7) to MYMV as compared to parents and standards at NIFA, Peshawar in kharif 2019 and 2020

Entry	Mungbean Yellow Mosaic Virus disease rating during					
	2019 at NIFA, Peshawar			2020 at NIFA, Peshawar		
	Percent infection	Score	Rating	Percent infection	Score	Rating
NBM-2-2-4-5	6.8	2	R	6.4	2	R
NBM-5-3-7	6.2	2	R	6.5	2	R
KBM	97.6	8	HS	91.2	8	HS
NBM	7.5	2	R	6.9	2	R
VC 1560 D	94.5	8	HS	96.2	8	HS
RAMZAN	8.2	2	R	7.6	2	R
NFM-2019	7.5	2	R	7.1	2	R

MYMV disease Score

Plant parts infected/ disease (%)	Score	Disease reaction
No infection	0	Immune (I)
1-5	1	Highly resistant (HR)
6-10	2	Resistant (R)
11-20	3	Moderately resistant (MR)
21-30	4	Tolerant (T)
31-40	5	Moderately tolerant (MT)
41-50	6	Moderately susceptible (MS)
51-80	7	Susceptible (S)
81 and above	8	Highly susceptible (HS)

Contemporary plant breeding emphasizes on breeding crop plants based on consumers' preferences so that a newly developed crop variety is readily adopted by the growers. The importance of crop breeding based on consumers' preferences has previously been reported elsewhere (Carlson, 2007; Wale and Yalew, 2007; Poudel and Jhonsen, 2009; Asrat et al., 2010; Blazy et al., 2011; Smith and Fennessy, 2011; Ward et al., 2013; Kassie et al., 2017). In Kuram, the growers prefer to grow mungbean with black seed coat color as this type of mungbean ensures better financial returns to the growers in the area. The newly developed black-seeded mungbean varieties NIFA Sikaram-21 and NIFA Spinghar-21 with high yield potential and consumers' preferred shiny black seed coat color are expected to play a vital role in uplifting the financial status of the growers of Kuram through improved economic harvest.

Controlling crop plants' disease through chemical applications is expensive and detrimental to the environment as well. The practical strategy to protect the potential yield of a newly developed crop variety is to induce genetic potential in the variety to resist diseases with no or little compromise on grain yield. In mungbean, resistance to MYMV disease is the accumulation of certain favorable genes with modifying effect (Khattak et al., 2000). As inherent disease resistance helps the genotypes to avoid yield losses (Oerke, 2006; Savary et al., 2012), NIFA Sikaram-21 and NIFA Spinghar-21 have the inherent potential to resist MYMV (**Table 7**) in order to protect their yield potential under disease conditions. MYMV does not prevail in Kuram due to un-availability of the vector, because of a relatively cooler environment during the growth cycle. However, as a result of the current climate change phenomenon, it is very likely that MYMV may occur in niches that are safe till now. NIFA Sikaram-21 and NIFA Spinghar-21 have the genetic potential to perform better under circumstances of MYMV incidence in Kuram.

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