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NARC-KALONJI: AN EARLY MATURING AND HIGH YIELDING VARIETY OF *NIGELLA SATIVA* RELEASED FOR CULTIVATION IN PAKISTAN

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Abstract

Kalonji (*Nigella sativa*) is an annual flowering plant investigated recently for its potential as a crop in Pakistan. NARC-Kalonji has been developed by the Institute of Agri-Biotechnology & Genetic Resources, National Agricultural Research Center (IABGR, NARC), Islamabad. A promising line was selected from indigenous germplasm collected from the Lahore area during 2002-2003 and was given the number MP120. After preliminary screening of 32 accessions, four elite lines were selected as promising genotypes on the basis of per row seed yield. These four accessions were grown over an area of 60 m^2 in plots during 2003-2004 using standard cultural practices. Significant differences in per acre yield were observed among the four accessions. Based upon two years of field evaluation at NARC, Islamabad, accession MP120 was selected for further testing for its seed yield and oil contents. Seed yield of NARC-Kalonji ranged between 290 and 333 kg/acre under field conditions at NARC, Islamabad. Seed of this accession was multiplied and trialed in diverse areas of Punjab before it was recommended as a new variety. The performance of the variety was assessed in the irrigated areas of Bahawalpur, Bhakkar, Faisalabad, Hasilpur, Lahore, Multan, and Vehari under adaptability trials conducted between 2005 and 2007. Seed yield at farmers' fields varied from 235 to 370 kg/acre during the last three years of testing at various sites. Although fertilizer application had a positive effect on biological yield, there was no significant effect on the economic yield. It was observed that Kalonji could be successfully cultivated in most parts of the country during the months of October and November.

Introduction

The age-old practice of using plant resources in traditional medicines is still in existence in the rural areas of Pakistan. Medicinal and aromatic plants are associated with the livelihood of a majority of rural people as they provide additional income. The trade in medicinal plants is an important source of revenue to the government. *Nigella sativa* L., is an annual aromatic plant native to Southwest Asia and the Mediterranean region. Its cultivation has been traced back more than 3,000 years to the kingdom of the Assyrians and ancient Egyptians (Khan, 2009). Presently, it is cultivated in various parts of the world, including Asia, the Middle East and North Africa. It belongs to family Ranunculaceae. The species is generally a short-lived annual, herbaceous plant. The height of the plant is approximately 20-60 cm. It possesses grayish green linear leaves that are wispy and thread-like. The flowers are delicate, pale blue or white, with a variable number of sepals and 5-10 petals that are about 2.5 cm wide (Muschler, 1912; Khan, 1999; Ahmad & Ghafoor, 2007). The flowers are distinguished by the occurrence of nectaries. The gynoecium consists of varied number of multi-ovulate carpels, which develop into follicles after pollination. The fruit is large and inflated, with 3-7 integrated follicles, each one with numerous seeds. Seeds are normally small (1-5 mm long), black or dark-grey with a rough grooved surface and an oily white interior (Khan, 1999; D'Antuono *et al*., 2002; Benkaci-Ali *et al.*, 2007). They are roughly triangular and possess a strongly pungent smell. They contain about 21% protein, 35% carbohydrates and 35-38% plant fats and oils (Anon., 2006; Ahmad & Ghafoor, 2007). The seeds have an immense medicinal value and are known to have numerous medicinal properties, mainly in the Unani-Tibb/Greco-Arab and Ayurveda systems of medicine (Abdulelah & Zainal-Abidin, 2007). The plant is usually known as black seed/black cumin (in English), Siyah-Daneh (in Persian), Kalonji (in Hindi and Urdu) and Habbat-ul-Barakah or Habbat-ul-Sauda (in Arabic).

Nigella sativa contains more than 100 valuable elements. It is an important source of protein, essential fatty acids and various vitamins such as A, B, B_2, C and niacin. It also contains minerals like calcium, iron, magnesium, potassium, selenium and zinc. Kalonji is used in food and medicines in many countries including Egypt, India, Iran, Pakistan, Saudi Arabia, and Syria. It is widely utilized in conventional medicine for curing a variety of respiratory and gastro-intestinal ailments in the entire Islamic world (Riaz *et al*., 1996). A number of researchers have extensively examined its composition and properties or reviewed the results of research on the species (Riaz *et al.*, 1996; Siddiqui & Sharma, 1996; Worthen *et al*., 1998). Kalonji has been used by millions of people in Southeast Asia, the Middle East and Africa to improve general health and fight various diseases. It has been used for a variety of conditions related to respiratory health, stomach and intestinal complaints, kidney and liver function, circulatory and immune system support, and rheumatism and associated inflammatory diseases (Malhotra, 2006; Ramadan, 2007). Seeds are also utilized for enhancing milk production in nursing mothers, promoting digestion and fighting parasitic infections. Kalonji seeds and/or their extracts have antidiabetic (Fararh *et al*., 2002), antihistaminic, antihypertensive, anti-inflammatory (Hajhashemi *et al*., 2004), antimicrobial, antitumor (Khan *et al*., 2003) and insect repellent effects (Fisher, 2002). Inhalation of its volatile oils is useful to treat chronic colds. It is useful in paralysis, facial palsy, migraine, amnesia and palpitations. The oil of Kalonji is effective in treating skin conditions like eczema and boils. The oil of the seeds is also effective for treating earaches (Khan, 1999; D'Antuono *et al*., 2002; Iqbal *et al*., 2011). Seeds are also used for flavoring and seasoning bread, pickles, and bakery products (Ramadan & Morsel, 2002). In Islam, it is regarded as one of the greatest forms of healing medicine available. The prophet Muhammad once stated that the black seed can heal every disease-except death. Kalonji therefore appears to be a potential multi-purpose crop of possible interest in Pakistan.

Kalonji has been extensively utilized for a variety of purposes in Pakistan, but its production has not been reported on commercial scale in the country (Iqbal *et al.*, 2009). Due to an increased demand for medicinal plants, it is a potential species for crop diversification to reduce risks of crop failure and improve crop productivity and income generation, especially in smaller land holdings (Ahmad *et al.*, 2004). The Institute of Agri-Biotechnology & Genetic Resources, National Agricultural Research Center (IABGR, NARC) initiated work on the collection, evaluation, preservation and sustainable use of medicinal plants in the country. The Kalonji germplasm collected and/or acquired from diverse sources was characterized to study the potential of this crop in Pakistan. In the framework of research programs on 'Introduction of Medicinal Herbs & Spices as Crops', and 'Production of Medicinal Herbs in Collaboration with Private Sector', the potential of Kalonji production has been explored in Pakistan and an early maturing, high yielding variety has been developed for general cultivation in the country.

Materials and Methods

Experimental material comprised 32 accessions of *Nigella sativa* germplasm collected from different eco-geographical areas of Pakistan along with USA, Ukraine, Iran, Afghanistan and India (Table 1). The germplasm was planted at NARC, Islamabad (33° 43' N and 73° 06' E) under field conditions during 2002-2003. The experiment was laid out in an augmented design. Row to row distance was kept at 50 cm. All the accessions were evaluated for various quantitative and qualitative traits. For most of the quantitative traits, five plants were selected at random from each accession and data were averaged for individual traits. Four genotypes, namely MP065, MP120, MP269, and MP354, performed well and were selected as potential lines for further use on the basis of their high yield. The identified accessions were grown over an area of 60 m^2 in the next growing season (2003-2004) using common production practices. Finally, the MP120 line was identified as a potential genotype for release as a new variety. Performance of the selected line was further tested in the irrigated areas of Punjab (Bahawalpur, Bhakkar, Faisalabad, Hasilpur, Lahore, Multan, and Vehari) under adaptability trials conducted between 2005 and 2007. Experiments were also conducted on the effects of fertilizer application and spacing on the yield of *Nigella sativa* using different doses of nitrogen and phosphorus, and various row spacing. Two selected genotypes were sown with three replications using three row spacings of 20, 30, and 40 cm, whereas fertilizer was applied at 100:100:00 (N:P:K). The seeds were planted in a split-split plot design, where genotypes and fertilizer application were kept in main plots and row spacing in sub-plots. Oil contents (percentage) were estimated following the standard methods by NMR and calibrated both at point one and point two by comparing with mustard oil standards (Anon., 2005). Data on seed yield and other related traits were recorded and statistically analyzed for descriptive statistics (Steel & Torrie 1984).

Table 1. Evaluation and characterization of Kalonji germplasm at NARC, Islamabad in 2002-2003.

No.	Site of collection	DF (50%)	Flower color	Plant color	Plant size	Plant type	Plant height (cm)	Yield /row (g)
1.	Sahiwal	144	Off white	Dark green	Small	Prostrate	30.5	72.2
17.	Islamabad	141	Light purple	Dark green	Medium	Prostrate	46.0	38.5
23.	Ghakkar Mandi	141	Pink	Light green	Tall	Erect	62.9	78.6
32.	Faisalabad	144	Light purple	Light green	Tall	Erect	76.5	40.1
41.	Shakargarh	162	White	Light green	Tall	Prostrate	64.9	28.2
48.	Kahuta, Rawalpindi	147	Light purple	Light green	Tall	Prostrate	42.9	39.6
65.	Kohat	164	Light purple	Light green	Tall	Prostrate	68.5	82.3
76.	Faisalabad	165	White	Light green	Tall	Prostrate	64.2	40.8
87.	Rawalpindi	146	Purple	Light green	Tall	Prostrate	65.5	48.3
102.	Lahore	135	Off white	Light green	Medium	Spreading	55.9	77.7
111.	Dera Ismail Khan	173	White	Light green	Tall	Spreading	82.5	60.6
119.	Lahore	150	Light purple	Light green	tall	Spreading	53.2	76. 6
120.	Lahore	150	Off white	Light green	Medium	Prostrate	36.2	108.5
171.	Chilas	165	Light purple	Light green	Tall	Prostrate	61.7	29.0
191.	Peshawar	172	Blue	Light green	Tall	Prostrate	72.3	49.4
202.	Dera Ismail Khan	174	Off white	Light green	Medium	Prostrate	80.3	20.3
216.	Mirpur, AJK	150	Blue	Light green	Medium	Prostrate	52.7	49.4
223.	Hasilpur	147	Light purple	Light green	Medium	Prostrate	56.2	20.3
245.	Mianwali	145	Light purple	Light green	Tall	Erect	67.3	33.0
262.	Chakwal	175	Blue	Light green	Tall	Erect	81.2	27.7
263.	Peshawar	137	Light purple	Light green	Medium	Spreading	59.5	35.4
269.	Faisalabad	175	Light purple	Light green	Medium	Spreading	59.5	109.7
340.	Rawalpindi	153	Light purple	Light green	Medium	Prostrate	43.5	69.8
354.	Qarshi Industries-Hatter	162	Light purple	Light green	Tall	Prostrate	72.4	108.9
355.	Faisalabad	172	Blue	Light green	Tall	Erect	84.3	10.3
358.	Islamabad	151	Purple	Light green	Tall	Erect	72.3	53.8
359.	Wah Cant	165	Blue	Light green	Small	Erect	80.9	40.5
362.	Islamabad	155	Blue	Light green	Tall	Prostrate	59.7	40.4
363.	Pindigheb, Attock	151	Light purple	Light green	Medium	Erect	43.2	35.1
36.4	Lahore	165	Purple	Light green	Tall	Prostrate	82.9	43.1
36.5	Multan	165	Purple	Light green	Tall	Prostrate	74.5	56. 5
36.6	Jund, Attock	168	Blue	Light green	Tall	Erect	76.2	45.9

DF = Days to flowering

Results and Discussion

Limited research work has been done on Kalonji in Pakistan to promote its cultivation at farmers' fields. Our goal was to collect, evaluate and identify elite lines on the basis of morphological and biochemical characteristics. The NARC-Kalonji is a local selection from germplasm collected from Lahore during 2002-2003. It has been developed by IABGR, NARC, Islamabad. On the basis of the first year preliminary trial, 4 genotypes, namely MP065, MP120, MP269, and MP354, performed well and were selected as potential lines for further use on the basis of their high yield (Table 1). Seed yield of the 4 accessions corresponded to the yield results of 2002-2003, indicating that these were diverse genotypes and had excellent yield potentials. A considerable level of variation was observed in the per acre yield of 4 accessions (Table 2). However, limited variation was observed in 1000-seed weight and oil contents of the accessions. The 1000-seed weight of the selected accessions varied from 2.2 to 2.3 g, while oil contents ranged between 33.75 and 34.40 percent. Based upon 2 years of field evaluation at NARC, two accessions, MP120 and MP269, were selected for their high yield and oil contents. Seed of these accessions was multiplied and tested at different localities from 2005-2006 to 2007-2008 in various regions of Punjab. NARC-Kalonji takes about 130-142 days to flowering. Plant height ranges from 74.6-90.5 cm. The plants mature in 185-190 days and the stem is hairy with a tendency to branch. Leaves are semi-erect, green and glabrous. Flowers are white with yellow stamens. The number of branches varies from 20-30 per plant. Follicles are medium and length ranges between 10.4-11.5 mm, while width varies from 8.99-10.59 mm. Seeds are matte, oval and deep black in color. The 1000-seed weight ranged from 2.23-2.80g, while oil content was 34%. Seed yield of NARC-Kalonji varied between 290-333 kg/acre under Islamabad conditions (Table 3).

Table 2. Performance of selected lines of Kalonji at NARC during 2003-2004.

S. No.	Accession No.	Seed yield (kg/acre)	Seed yield per 5 m row during 2002-2003	Oil contents (%)	1000-seeds weight (g)
1.	MP065	273.2 ± 1.73	82.3 ± 1.65	33.78 ± 0.38	2.3 ± 0.09
2.	MP120	333.2 ± 2.96	108.5 ± 1.19	33.94 ± 0.20	2.2 ± 0.08
3.	MP269	330.0 ± 2.38	109.7 ± 1.53	34.40 ± 0.40	2.3 ± 0.09
4.	MP354	300.0 ± 1.83	108.9 ± 1.18	33.75 ± 0.35	2.3 ± 0.08

Note: Data is based on averages of four replications, ± indicates standard errors of the means

Table 3. Salient Features of NARC-Kalonji.

No.	Trait	Accession (MP120)		
	Quantitative traits (Avg. values)	**2002-2003**	**2003-2004**	**2004-2005**
1.	Days to flower initiation	137	130	142
2.	Days to 50% flowering	150	165	157
3.	Days to maturity	190	195	185
4.	Plant height (cm)	90.5	74.6	88.1
5.	No. of branches per plant	26	20	30
6.	No. of follicles per plant	201	207	198
7.	Follicle length (mm)	10.9	11.5	10.4
8.	Follicle width (mm)	8.99	10.59	9.89
9.	Follicle weight per plant (g)	30.31	28.84	24.48
10.	1000-seed weight (g)	2.80	2.23	2.24
11.	Seed yield per plant (g)	8.83	9.22	8.75
12.	Seed yield per row (5 m)	92.9	84.7	78.9
13.	Seed yield per acre (kg)	290	333	235
14.	Oil contents of seed (%)		33.94	
	Qualitative traits:			
15.	Growth vigor		Good	
16.	Hairiness		Hairy	
17.	Flower color		Off-white	
18.	Plant color		Light green	
19.	Plant type		Prostrate	
20.	Plant size		Medium	
21.	Seed color		Black	

During the last few years, it has been observed that Kalonji can be successfully cultivated in most parts of the country during the months of October-November (Table 4). Although fertilizer application exhibited positive effects on biological yield, this did not translate into increased economic yield. From our experiments conducted at NARC, it has been observed that the best row spacing for obtaining maximum biological yield, as well as seed yield, is 30 cm; this resulted in seed yield of 788 kg per hectare. As Kalonji must be cross-pollinated, insects or honeybees may also play an important role in yield enhancement. The crop is attacked by a borer if harvesting is delayed. However, if the crop is harvested in time after reaching physiological maturity, borer attacks could be minimized or curtailed completely. Seed yield at farmers' fields varied from 235-370 kg/acre at various locations during three years of testing (Table 5).

Table 4. Production technology of NARC-Kalonji.

Cultural practice	Requirements
Soil:	Fertile; Mera soil
Soil preparations:	3-4 ploughings along-with Suhaga
Time of sowing:	October to November
Method of sowing:	Broadcasting; Line sowing; Ridges
Seed rate:	4-5 kg/acre
Row spacing:	1½ feet (45 cm)
Irrigations:	4 to 5 per growing season
Hoeing:	1-2 Weedings
Harvesting:	April to May
Potential yield:	Up to 400 kg/acre

Table 5. Adaptability study/yield performance of NARC-Kalonji at Farmers' Fields in Punjab.

S. No.	Name of growers and locations	Avg. Seed Yield (kg/acre)		
		2005-06	2006-07	2007-08
1.	Mrs. Shahida Doultana, Ahmadabad Model Farm, Vehari.	270	325	308
2.	Niaz Ahmed Bosan, Bosan Model Farm, Bosan Road, Multan.	235	-	-
3.	Fiaz Ahmed Warraich, Khanewal Road, Vehari.	255	-	-
4.	Jahan Khan Farm/Thatta Jabana Farm, Sargodha Road, Jhang.	280	-	-
5.	Waqar Ahmed, Chak No. 492/GB, Samundari, Faisalabad.	-	370	-
6.	Ch. Muhammad Nasrullah, Chak 60-61/ML, District Bhakkar.	-	237	-
7.	Taj Muhammad, Chak Fateh, Hasilpur, Dist. Bahawalpur.	-	260	-
8.	Muhammad Hayat, 43km Multan Road, Manga Mandi, Lahore.	-	-	289
9.	Saeed Ahmad, Bhaseen, Near Jallo More/BRB Canal, Lahore.	-	-	305

Fig. 1. Performance of NARC-Kalonji at PGRP-IABGR, Islamabad.

Fig. 2. Performance of NARC-Kalonji at farmer's field in Punjab.

In spite of its great importance, little attention has been paid to production improvement so Kalonji remained a minor or underutilized crop. Although it has been extensively studied for its medicinal properties, little work has been carried out on its genetic improvement and agronomic practices. Due to increased demand of Kalonji oil in trade for medicinal purposes, it is a potential plant species in crop diversification to reduce risks of crop failure and improve crop productivity and income generation, especially in smaller land holdings. Kalonji is cultivated during the winter season in much the same way as wheat. After going through various procedures and spot examination by the evaluation committee, line MP120 was finally recommended as a new variety 'NARC-Kalonji' in 2009 for general cultivation. Sufficient quantity of the seed of NARC-Kalonji is available at NARC, Islamabad and Punjab Seed Corporation (PSC) Farm, Khanewal.

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