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GERMPLASM SCREENING OF *Myrtus communius* VAR. *Italica* l. FOR CULTIVAR DEVELOPMENT FROM DIR LOWER (MALAKAND DIVISION), PAKISTAN

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Abstract

Habb-ul-Aas is a commercial drug obtained from *Myrtus communis* L and widely used for abdominal disorders in Pakistan. This sclerophyllous shrub is commonly found in the Lower Dir, Malakand Division, Pakistan. This plant is used as crude drug in herbal as well as pharmaceutical industry due to presence of aromatic oil obtained from various parts of the plant. Two varieties viz., *italic* and *baetica* of the plant exist in the world; however, no study reported before about the existence of this species up to the varietal level from Pakistan. The current study was undertaken to characterize the existing germplasm by using certain morphometric parameters in relation to physiognomic features. They exhibit high variability among characters, especially for characteristics useful in selecting cultivars for commercial production. Among the various ecotypes, MBT3 possessed high shoot length (29.96cm), leaf area (2.3cm), fruit volume (0.97ml) & fruit weight (0.88g) and pulp (0.87g) and therefore recommended for the cultivar development.

Keywords: Aromatic plants. Cultivar. Ecotypes. Germplasm. *Myrtus communis*. Sclerophyllous shrub.

1. Introduction

Myrtle (*Myrtus communis* L.) is a sclerophyllous shrub attaining to 30-35 feet that belongs to Myrtaceae family. The plant possesses small dark green leaves with white flowers and dark fruits. The plant is relatively frost resistant and is widely distributed in the Mediterranean region. It is popular due to presence of essential oil mostly extracted from leaves and mature fruits that is rich in linear, cyclic and bicyclic monoterpenes (Savikin-fodulovic et al. 2000). It is one of the popular drugs being used in the Unani system of medicine since the ancient Greece period. Various plant parts possess an essential oil which is used in cosmetics, flavor, drinks and extensively as therapeutics. The leaves used as a cooking spice (Rossi et al. 2009). The different parts of *M communis* such as branches, berries, and leaves have been used worldwide as a traditional/folk medicine for the treatment of various ailments and diseases (Mir et al. 2020). The essential oil is rich in bioactive monoterpenes and sesquiterpenes and their derivatives. As folkloric treatment, it is used for treating various diseases that validated by various pharmacological activities, such as antidiarrheal, antimicrobial, anti-diabetic, vasodilator, antispasmodic, antiulcer, anticancer, antioxidant, sedative-hypnotic, anxiolytic, and anti-inflammatory and others (Mekonnen and Gashaw 2017). Furthermore, leaves and fruit are used in traditional medicine as a hypoglycemic agent (Elfellah et al. 1984).

The fruits of this plant are mostly composed of volatile oils, tannins, sugars, flavonoids, and organic acids such as citric and malic acids (Martin et al. 1999). The oil contents of fruit include delphinidin,

petunidin, malvidin, peonidin and cyanidin-3-mono and 3, 5-diglucosides as well as glycosides of myricetin and quercetin. Furthermore, arabinoside derivatives of delphinidin, malvidin, petunidin and cyaniding were isolated from myrtle berries extracts. The leaves possess tannins, flavonoids such as quercetin, catechin and myricetin derivatives and volatile oils (Romani et al. 1999). Various studies have demonstrated antioxidant, insecticidal, antimicrobial anti-inflammatory from the essential oils (EOs). The chemical composition of EOs indicated the presence of α-pinene, 1,8-cineole, methyleugenol, eugenol and myrtenyl acetate (Aicha et al. 2019).

Dir hills are located in Hindukush range, Pakistan which endowed with clumps of forest of *Myrtus communis* and authors (Rahim et al. 2012) previously reported *Myrtus communis* var. *baetica* (locally called *Spin Mano*) from the study area. After detailed screening, another variety *italica* (locally called *Tor Mano*) was determined. This variety has morphometric variations, therefore need to evaluate existing germplasm in order to demark clear lineage with reference to essential oil and fruit production used in herbal industry. Since the plant as well as its varieties has commercial importance, therefore 104 wild growing myrtle germplasm from the area were evaluated using the most valuable characters such as vegetative, organoleptic, and reproductive characters for cultivar development.

2. Material and Methods

The study area

Geographical Location: Dir is located in the Khyber Pukhtoonkhwa Province of Pakistan. It lies in Hidukush hilly range between 35° 10 to 35° 16 N and 71° 50 to 71° 83 E (Figure 1). The elevation of District ranges from 1200m to 2800m above sea level. This whole area was the part of state (5284 km²), now divided into two districts of Pakistan - Upper Dir and Lower Dir covering an area of 15,85km. In the North of Lower Dir, there are mountainous peaks rising from 1100 to 3119 meter. Dir have small streams rising from sub valleys to join the main Panjkora River at different locations (Hazrat et al. 2011).

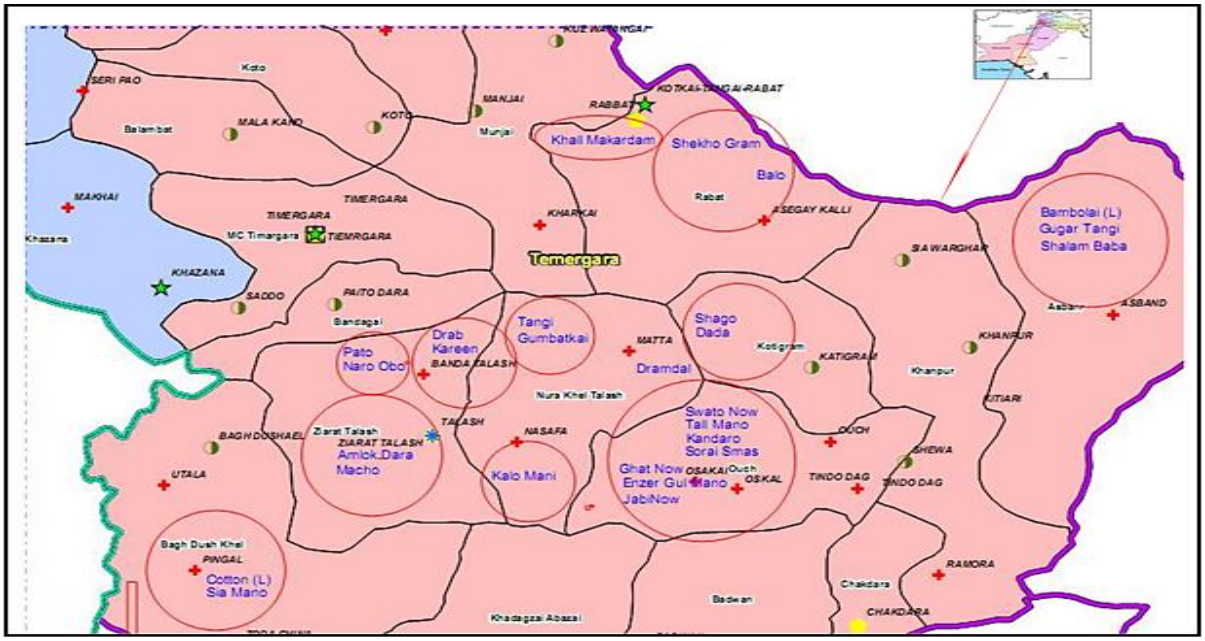

Figure 1. Map of the study area showing the sampling sites.

Climate: Depending high altitudes, the area has prolonged cold season except the month of July in which temperature may be 15.67 to 32.52°C. January and February are the coldest months, and the temperature generally falls below freezing point. The mean maximum and minimum temperature during these months remain 11.22°C and 2.39°C respectively. The winter season starts from mid-November and remain until the end of March. The relative humidity was found higher in January, February, August, and December (Hazrat et al. 2011). During the month of March, the area receives highest rainfall i.e. 242.22 mm.

The snowfall starts from December to March and remains up to June and July on high mountains, adding to the beauty of the valley (Hazrat et al. 2013).

Plant specimen collection: The entire study area was widely explored for the collection of plant specimens having different populations during October to December 2011 at the time of fruitification period. During collection, 52 representative sites distributed at different altitudes such as Osakai (Swato Nawo, Jabi Nawo, Ghat Nawo, Tall Mano, Enzer Gul Mano, Khandaro, Sorai Smas, Tall Mano), Bambolai (Jabi Tangi, Khat Chena, Mano Tangi), Gujar Tangi (Mian Banda Tall, Gujar Tangi, Shadgano Seri, Shahalam Baba, Doop, Baghgai), Talash (Tangi, Shavy Tangi, Banda, Kareen, Darb, Gumbatkai, Pato, Narowobu, Kalo Manai), Nasafa (Zulan Gut), Macho (Rung Banda, Narai Manzai),Arakh, Am look Dara, Talash Dushkhel, Cotton lower (Kotai Jai, Yagi Tall, Naranjano Khwar, Tatodund Mano, Purkha,Siah Mano), BaghDushkhel (Ser Mano), Adenzai (Khairabad, Dada, Zaman Patai, Shago, Dada), Khal (Makar Dam) and Rabat (Balu) were selected from the study area. The ecotypes were coded according to area of collection. Different physiognomic features of vegetation were also recorded for the identification of sampling sites. The fruits of wild growing myrtle were harvested manually from 52 accessions found in each site situated in the Lower Dir region of Pakistan. The fruit colors of all accessions were mostly dark and dark red. Voucher specimens were prepared using traditional method of pressing, drying and mounting on standard herbarium sheets and deposited in Botany Department for upcoming reference.

Branch samples of each ecotype were brought to the Taxonomy & Phytomedicine Lab., Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan for biometric and Qualitative analysis. Some of the characters which were likely to be lost during voucher preparations were recorded at the sampling sites. A digital camera was used for capturing images to show habit of specimens. Global position system (GPS) was used to record coordinates of different ecotypes as well as altitudinal gradients at the sampling site. For analysis, we have collected specimens from the mature plants (approx. 4-5 years old) each having 3-replicates samples were collected for biometric and morphological analysis of shoot length, number of internodes per shoot, internode length and number of berries per shoot randomly three replicate samples of leaves were measured for leaf length and width. For the evaluation of fruit characteristics, mature berries were harvested from each of the three randomly selected shoots of 150 fruit were selected for fruit weight, volume, length, number of seeds, weight of seeds, width, calyx diameter, length of peduncle and color. Fruits and their seeds were weighed using a balance with a precision of ± 0.01g. The fruits size was measured using a digital caliper. Flowering period, ripening time and species features were recorded personally that was confirmed by asking from the local inhabitants at different sites on the spots.

Statistical analysis

PAST program Version 2.02 (Hammer 1999-2010) was used for statistical application of all recorded biometric values to depict variability and to draw associations amongst biometric determinations from the selected ecotypic populations.

3. Results

The myrtle ecotypes were collected from various growing locations of Dir Lower (Malakand Division) that lies in southern parts of Pakistan (Figure 1). The highest canopy cover was observed in TST1 (14.6m) followed by TNO5 (9m), while the lowest canopy was noted in CSM4 (0.5m), RBU3 and TTG5 (1m each). The highest height of the ecotypes was in TST1 (12.3m), TNO1, TNO4 and TNO5 (10m each), while on the other hands the ecotypes such as CSM4 and TTG5were 1-1.5m tall. The collected ecotypes possessed a range of altitudinal variations from 848 to 1734 meter. Textural classes seem to be variables and indicates that most of ecotypes preferred to grow in This species possessed variability in adapting different soil texture classes; however, it is observed that most of the ecotypes suited well in moist soils found in valley bottoms, streams, springs, hill slopes at the tops of lofty crags and field borders. I was revealed that myrtle is sand loving plant and most of the ecotypes were preferably growing in loamy and sandy soils (22.12 and 20.19% respectively), followed by coarse sand (16.35%), fine sandy loam (10.58%) and fine to medium fine sand (9.62 to 8.65%).

The calyx always found persistently attached with fruit, with the prominently closed, partially open or open shaped. Closed, open and partially open shapes shared equal proportion (34-35 nos each). There was good diversity of fruit shapes, and 10 different shapes were identified from the collected ecotypes. The berry shape was found highly variable and 10 different shapes were recorded from the selected ecotypes (Figure 2). The shapes were in the order of spherical (18.27%)> elongate (17.31%)> obovate (14.42%)> turbinate (13.46%)> pyriform (11.54%)> flat (8.65%)> elliptic (7.69%)> round (3.85%)> oblong (2.88%)> and globose (1.92%). Fruit peel was typically dark color and majority of the ecotypes i.e., 61 out of 104 ecotypes (58.65%) had this shade, whereas rest of the ecotypes had equally dark blue to dark red tinged fruits.

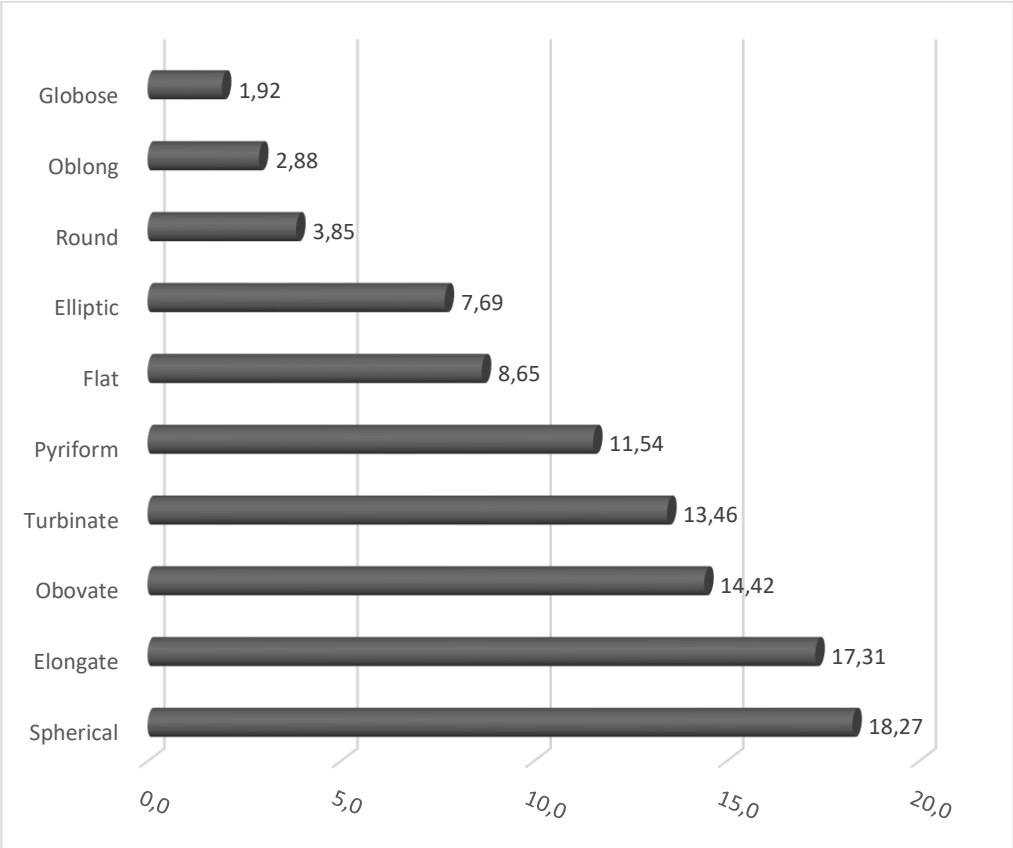

Figure 2. Fruit shapes of different ecotypes of myrtle from Malakand, Pakistan.

There were three seed shapes identified from selected ecotypes. There was slight difference in fruit color and most of the ecotypes possessed dark color (61 nos and 58.65%), followed by dark blue and dark red (21.15 and 20.19% respectively). Kidney and snail-shapes were found in equal proportional (39 each) followed by bean-shaped (26 ecotypes).

There was good variability in terms of fruits per shoot amongst the ecotypes. These may be due to differences in climate and soil types. It has been observed that ecotypes growing in Loams and rocky substrate possessed good average of fruits per shoots. Highest fruits/shoot were recorded from CSM5 (36), followed by TTG4 (35), TNO5, TTG8, OJN1, DKJ1 (33), OJN2, OJN6 (31 each), BJT1 (30), TBA2 (29), SBD1 (28), TMC4, SBG4, OOS13 (27 each), TNO1 (26), TTG9 and OOS7 (25 each). While ecotypes such as TMNI, CSM6, OSN1, TTG5, OOS10, TMC5, TKM1, TAK2, OTM4 and BKC1 possessed 1.1, 1.9, 2.5, 2.7, 3.3, 3.6 and 3.8 fruits per shoot respectively (Table 1).

The ripening of fruits occurred during October to December. The early ripening ecotypes are found growing at sunny focal areas such as Banda Tangi, KareenDrab, Pato, Gumbatkai, Narowobu, Shago, Bambolai, Shahlam Baba, Rabat, Khalas well as in Dushkhail areas of Baugh and lower cotton. The late ripening ecotypes located at hillocks and relatively in high altitudinal areas such as Macho (Rung Banda). Due to variations in north and south facing slopes, fruit ripening of ecotypes from the same sampling areas, however could be highly differentiated as in the case of ecotypes selected from the sample areas at Osakaiand Nasafa and as well as Macho.

Amongst the vegetative characters, shoot length, internodes (Nos), internodal length, leaf length, leaf width, leaf length/width ratio, peduncle length and calyx diameter were selected to seek variations in selected ecotypes. The shoot length was varied between 4.08 cm and 30 cm with a mean of 14.20 cm (Table 1). The highest shoot length depicted vegetative vigorness such as those of ecotypes OOS13 (30cm), MBT3 (29.96 cm) and OTM3 (28 cm), whereas shorter lengths were distinctive features of dwarf or weak ecotypes found in TTG1 (4.08 cm), TTG5 (4.09 cm), TTG9 and CPU (4.15 cm each). The internode numbers were varied from 4.5 cm to 28 cm with a mean of 13.92 cm. The largest internodes were recorded in BJT1 with 28 number, followed by OJN4 (27), OTM2 (24.2) and TAK1 (23.5), while TBA1, TNO3 possessed least internodes (4.5), followed by TTG9 and CPU (4.6 each). The intermodal length was ranged from 0.4to 1.8 cm with a mean of 1.06 cm. The ecotype labeledTBA1 possessed highest length (1.8 cm) that was followed by TBA2, CYT1, BSM1, BMT1, OOS7, OTM5 and OOS13 each one having 1.7 cm. But, OJN4 had least height (0.4 cm), followed by CTM, TDB1 and OOS6 with each of 0.5 cm.

Table 1. Morphometric values of selected ecotypes from Dir Lower.

S. No	Ecotype	Vegetative Biometry							Floral Biometry						
		SL	I.	I.l	L.l	L.w	L.l/w	Fr/Sh	Fr.l	Fr.w	Fr.l/w	Fr.v	Fr.we	P	Se.w
1	TTG1	4.08	5.8	0.7	2.8	1.7	1.64	13	1.1	0.8	1.37	0.36	0.27	0.14	0.13
2	TTG2	12	11.8	1	4.3	2.5	1.72	7.8	1.2	0.7	1.71	0.59	0.47	0.36	0.11
3	TTG3	6.06	6.7	0.9	3.3	1.1	3	17	1.1	1	1.1	0.22	0.17	0.05	0.12
4	TTG4	10.16	7.8	1.3	3.2	1.3	2.46	11	1.1	0.9	1.22	0.36	0.26	0.17	0.09
5	TTG5	4.09	5.8	0.7	2.8	1.6	1.75	10	1.2	0.6	2	0.68	0.57	0.46	0.11
6	TTG6	13	11.8	1.1	4.3	2.5	1.72	19	0.9	0.7	1.28	0.65	0.53	0.43	0.1
7	TTG7	8.07	6.7	1.2	3.2	1.1	2.9	14	1.1	0.9	1.22	0.51	0.42	0.29	0.13
8	TTG8	11	7.8	1.4	3	1.3	2.3	12	1.1	0.7	1.57	0.55	0.45	0.36	0.09
9	TTG9	4.15	4.6	0.9	2.7	1.2	2.25	23	1.1	1	1.1	0.44	0.34	0.22	0.12
10	TBA1	8.2	4.5	1.8	3.1	1.3	2.38	13	0.8	0.6	1.33	0.71	0.65	0.5	0.15
11	TBA2	15.5	9	1.7	2.4	1.2	2	26	1.3	1.1	1.18	0.65	0.56	0.44	0.12
12	TGU1	16.5	10.2	1.4	3.8	1.4	2.71	15	1.1	0.8	1.37	0.62	0.52	0.43	0.09
13	TGU2	14.29	12.5	1.2	2.8	1.1	2.54	21	1.3	1	1.3	0.51	0.42	0.33	0.09
14	TGU3	9	7.8	1.1	2.5	1.4	1.78	19	0.9	0.7	1.28	0.57	0.46	0.34	0.12
15	TPO1	12	10	1.2	3.4	1.4	2.42	10.5	0.9	0.6	1.5	0.56	0.48	0.35	0.13
16	TNO1	20.03	15.4	1.3	4.1	1.8	2.27	29	1.1	1.2	0.91	0.51	0.44	0.34	0.1
17	TNO2	15.3	17	0.9	2.7	1.2	2.25	33	0.8	0.8	1	0.38	0.29	0.19	0.1
18	TNO3	5.9	4.5	1.3	3.2	1.3	2.46	27	1.1	0.7	1.57	0.41	0.34	0.26	0.08
19	TNO4	19.3	12	1.6	2.6	1.2	2.16	36	1.2	0.8	1.5	0.42	0.35	0.22	0.13
20	TNO5	12.27	10.2	1.2	3.8	1.4	2.71	25	0.8	0.7	1.14	0.35	0.27	0.16	0.11
21	TNO6	18	15	1.2	2.8	1.1	2.54	23	1.3	0.8	1.62	0.52	0.44	0.04	0.4
22	CYT1	13.26	7.8	1.7	2.3	1.3	7.66	6.7	0.9	0.9	1	0.55	0.46	0.44	0.02
23	CYT2	11	8.8	1.2	3.4	1.4	2.42	3.3	0.9	0.7	1.28	0.49	0.41	0.3	0.11
24	CTM	10.6	21	0.5	4.2	1.7	2.47	1.9	0.8	0.6	1.33	0.86	0.75	0.58	0.17
25	CPU	4.15	4.6	0.9	2.9	1.2	0.22	5.7	1.3	1.1	1.18	0.38	0.31	0.21	0.1
26	CSM1	7.2	9	0.8	3	1.3	2.3	13	0.9	0.7	1.28	0.9	0.79	0.67	0.12
27	CSM2	14.5	16	0.9	2.5	1.2	2.08	14	1.2	0.8	1.5	0.94	0.84	0.71	0.13
28	CSM3	24.2	22	1.1	2.8	1.3	2.15	19	1.1	1.1	1	0.92	0.81	0.65	0.16
29	CSM4	11.78	16.8	0.7	2.3	1.3	1.76	18	0.9	0.6	1.5	0.89	0.8	0.66	0.14
30	CSM5	8.66	9.6	0.9	2.4	1.2	2	24	1.3	1.1	1.18	0.84	0.75	0.58	0.17
31	CSM6	5.85	8.3	0.7	2.9	1.3	2.23	20	0.9	0.7	1.28	0.81	0.76	0.64	0.12
32	CNK	18	21.8	0.8	3.2	1.4	2.28	8.3	1.2	0.8	1.5	0.53	0.46	0.29	0.17
33	BSM1	16	9.4	1.7	3.5	1.3	2.69	7.9	1.1	1	1.1	0.54	0.44	0.37	0.07
34	TKN1	12.75	21.2	0.6	2.8	1.2	2.33	6.9	1.3	1.2	1.08	0.38	0.31	0.21	0.1
35	TDB1	9.8	19.4	0.5	4.2	1.8	2.33	17	0.9	0.6	1.5	0.51	0.37	0.25	0.12
36	TTG10	22.97	16.4	1.4	3.3	2.5	1.32	6.3	1.2	0.9	1.33	0.53	0.41	0.26	0.15
37	DKJ1	10	9.5	1	3.2	1.1	2.9	4.4	1.2	1	1.2	0.38	0.31	0.11	0.2
38	GTN1	14.7	13.3	1.1	2.6	1.3	2	11	0.9	0.8	1.12	0.51	0.45	0.29	0.16
39	GTN2	21.6	18	1.2	2.9	1.2	2.41	16	1.2	1	1.2	0.38	0.31	0.17	0.14
40	BJT1	19.7	28	0.7	3.1	1.1	2.81	4.2	1.3	1	1.3	0.49	0.44	0.32	0.12
41	BKC1	17.45	21.8	0.8	3.1	1.1	2.81	4.9	0.9	1	0.9	0.47	0.38	0.37	0.01

S. No	Ecotype	Vegetative Biometry						Floral Biometry							
		SL	I.	I.l	L.l	L.w	L.l/w	Fr/Sh	Fr.l	Fr.w	Fr.l/w	Fr.v	Fr.we	P	Se.w
42	BMT1	16	9.4	1.7	3.5	1.3	2.69	8.1	1.2	0.8	1.5	0.55	0.46	0.33	0.13
43	MBT2	16.2	20	0.8	3	1.2	2.5	33	1.2	1	1.2	0.96	0.87	0.75	0.12
44	MBT3	29.96	21.4	1.1	4.3	1.8	2.38	35	1.3	1.1	1.18	0.97	0.88	0.76	0.12
45	SBD1	22	17	1.3	2.9	1.3	2.23	4.6	0.9	0.7	1.28	0.73	0.61	0.45	0.16
46	SBG2	17.1	16.8	1	3.4	1.4	2.42	5.9	1.2	0.9	0.22	0.49	0.44	0.24	0.2
47	SBG3	21.8	15.5	1.4	3.7	0.9	4.11	7.2	1.3	0.8	1.62	0.49	0.43	0.03	0.4
48	SBG4	19	14.3	1.3	3.1	1.2	2.58	8.6	0.9	0.5	1.8	0.51	0.40	0.87	0.13
49	TAK1	14.2	23.5	0.6	2.5	0.8	3.12	10.5	1.2	0.6	2	0.46	0.37	0.26	0.11
50	TAK2	14.5	16	0.9	2.8	1.1	2.54	2.7	1.1	1	1.1	0.49	0.44	0.35	0.09
51	TAD	12	11.9	1	2.3	1.2	0.19	3.6	1.1	0.7	1.57	0.49	0.42	0.28	0.14
52	TMC1	19.2	16	1.2	2.5	1.3	1.92	33	1.3	0.8	1.18	0.51	0.37	0.27	0.1
53	TMC2	12.7	14	0.9	2.9	1.2	2.41	27	1.2	1.1	1.09	0.7	0.59	0.30	0.7
54	TMC3	17.4	17.3	1	3.4	1.1	3.09	31	1.1	0.7	1.57	0.49	0.49	0.34	0.15
55	TMC4	21.75	14.5	1.5	3.2	1.3	2.46	25	1.3	0.8	1.62	0.51	0.36	0.25	0.11
56	TMC5	11.4	19	0.6	3.7	1.2	3.08	21	1.2	0.9	1.33	0.68	0.57	0.45	0.12
57	OJN2	13	11.4	1.1	2.7	1.3	2.06	8.4	1.1	0.7	1.57	0.49	0.43	0.3	0.13
58	OOSa	11.5	9.5	1.2	2.8	1	2.8	7.7	1.2	0.8	1.5	0.51	0.45	0.34	0.11
59	OJN3	13.1	11.9	1.1	2.3	1.2	1.91	6.9	1.2	0.5	2.4	0.74	0.65	0.53	0.12
60	OJN4	10.9	27	0.4	2.8	1	2.8	7.3	1.1	0.7	1.57	0.49	0.39	0.22	0.17
61	OJN5	24.45	22.2	1.1	2.9	1.2	2.41	6.3	1.4	0.5	2.8	0.52	0.46	0.33	0.13
62	OJN6	15	14.8	1	3.1	1.4	2.21	4.6	1.2	0.9	1.33	0.75	0.66	0.47	0.19
63	OOS1	16.5	16.5	1	3.6	1.1	3.27	7.3	1.3	0.7	1.85	0.49	0.37	0.27	0.1
64	OJN1	14.7	11.3	1.3	3.1	1.2	2.58	9.5	1.1	0.6	1.83	0.51	0.41	0.29	0.12
65	OSN1	15.77	14.3	1.1	2.6	1.3	2	4.9	1.3	0.5	2.6	0.73	0.63	0.48	0.15
66	OSN3	10.9	12.1	0.9	2.7	1	2.7	14	0.8	0.7	1.14	0.38	0.29	0.18	0.11
67	OOS2	11.68	11.9	0.8	2.6	1.2	2.16	4.8	1.1	0.7	1.57	0.41	0.39	0.26	0.13
68	OOS3	11	9.7	1.1	2.4	1.3	1.84	5	1	0.5	2	0.42	0.35	0.25	0.1
69	OOS4	18	17.9	1	2.9	1.1	2.63	5.3	0.8	0.8	1	0.35	0.27	0.18	0.09
70	OOS5	19.25	14.8	1.3	3.4	1.1	3.09	5.9	1.3	0.8	1.62	0.52	0.45	0.31	0.14
71	OOS6	10	19.5	0.5	3.8	1.3	2.92	6.2	0.8	0.8	1	0.38	0.29	0.23	0.06
72	OOS7	21	12.3	1.7	3.3	1.4	2.35	8.4	1.1	0.9	1.22	0.41	0.34	0.23	0.11
73	OOS8	14.8	21	0.7	2.3	1.3	1.76	6.4	1.3	1	1.3	0.56	0.47	0.34	0.13
74	OGN3	14.8	18.5	0.8	2.9	1.4	2.07	7.6	1.1	0.7	1.57	0.48	0.48	0.33	0.15
75	OTM1	13.1	11.9	1.1	2.6	1	2.6	6.6	0.9	0.5	1.8	0.51	0.39	0.25	0.14
76	OTM2	15.1	24.2	0.6	2.8	1.4	2	14	1.3	0.9	1.44	0.73	0.61	0.5	0.11
77	OTM3	28	21.4	1.3	4.3	1.5	2.86	19	1.1	0.8	1.37	0.56	0.46	0.27	0.19
78	OTM4	24.1	18.4	1.3	3.5	2.5	1.4	10	1.3	0.7	1.85	0.24	0.19	0.07	0.12
79	OTM5	16.15	9.5	1.7	3.2	1.1	2.9	11	1.3	0.8	1.62	0.29	0.23	0.1	0.13
80	OTM6	14.86	21.2	0.7	2.9	1.4	2.07	5.4	0.9	0.7	1.28	0.45	0.27	0.13	0.14
81	ONG1	13	11.7	1.1	3.3	1.2	2.75	7.8	0.8	0.6	1.33	0.33	0.26	0.14	0.12
82	OOS9	9.5	15.7	0.6	3.1	0.9	3.44	5.5	0.7	0.8	0.87	0.24	0.18	0.06	0.12
83	OOS10	12.6	11.3	1.1	2.7	1.2	2.25	4.7	1	0.8	1.25	0.3	0.25	0.16	0.09
84	OOS11	11	14.8	0.7	3.4	0.8	4.25	3.3	1.2	0.9	1.33	0.47	0.38	0.25	0.13
85	OOS12	12.15	13.5	0.9	3.2	1.3	2.46	6.9	1.4	0.8	1.75	0.24	0.17	0.1	0.07
86	OOS13	30	17.3	1.7	3.1	0.7	4.42	3.8	0.9	0.7	1.28	0.32	0.25	0.2	0.05
87	OOS14	16	10	1.6	2.5	1.1	2.27	2.5	0.9	0.7	1.28	0.45	0.37	0.29	0.08
88	TKM1	6.5	13	0.5	3.3	1.1	3	5.1	0.9	0.6	1.5	0.33	0.25	0.18	0.07
89	TST1	14.6	12.3	1.1	2.4	1.3	1.84	2.7	0.7	0.6	1.16	0.29	0.22	0.16	0.06
90	KMD1	12.2	11.1	1.1	2.5	1	2.5	1.1	1.2	0.9	1.33	0.47	0.37	0.25	0.12
91	KMD2	11.25	12.5	0.9	3.4	1.6	2.12	14	0.8	0.8	1	0.24	0.17	0.07	0.1
92	RBU1	16.6	15.1	1.1	2.6	1.5	1.73	28	1.1	0.7	1.57	0.88	0.77	0.56	0.21
93	RBU2	17.8	17.8	1	3.6	1.1	3.27	31	0.6	0.6	1	0.88	0.8	0.61	0.19
94	RBU3	13.5	12.3	1.1	2.7	0.7	3.85	33	1.3	0.7	1.85	0.91	0.83	0.62	0.21
95	RBU4	9.1	11.4	0.8	2.3	1.3	1.76	30	1.4	0.6	2.33	0.93	0.81	0.61	0.2
96	RBU5	12.9	18.5	0.7	3.4	1.7	2	27	1.3	1.1	1.18	0.85	0.76	0.59	0.17
97	NZG1	11.9	11.9	1	3.7	1.2	3.08	11	1	0.8	1.25	0.46	0.38	0.22	0.16
98	NZG2	7.2	12	0.6	2.7	1.3	2.06	6.9	1.3	1	1.3	0.51	0.44	0.35	0.09
99	TMC1	19.6	17.9	1.1	4.1	1.2	3.41	15	0.9	0.7	1.28	0.57	0.43	0.3	0.13

S. No	Ecotype	Vegetative Biometry						Floral Biometry							
		SL	I.	I.l	L.l	L.w	L.l/w	Fr/Sh	Fr.l	Fr.w	Fr.l/w	Fr.v	Fr.we	P	Se.w
100	TMNI	17.9	16.3	1.1	2.9	1.2	2.41	4.7	0.9	0.6	1.5	0.56	0.48	0.36	0.12
101	KAD1	11	15.8	0.7	3.3	1.8	1.83	3.3	1.2	0.6	2	0.47	0.38	0.3	0.08
102	KAD2	11.2	11.2	1	4.3	2.5	1.72	6.9	1.2	0.9	1.33	0.47	0.42	0.31	0.11
103	SSO1	10.06	6.7	1.5	3.3	1.1	3	5.5	0.7	0.8	0.87	0.39	0.33	0.22	0.11
104	SSO2	13.1	7.8	1.6	3.2	1.3	2.46	4.7	1	0.8	1.25	0.28	0.23	0.15	0.08

SL=Shoot Length, I.= Internode, I.l= Internodal length, L.l= Leaf length, L.w= Leaf width, L.l/w= Leaf length/width, Fr/Sh= Fruit/shoot, Fr.l= Fruit length, Fr.w= Fruit width, Fr.l/w= Fruit length/width, Fr.v.= Fruit volume, Fr.we= Fruit weight, P= Pulp, Se.w= Seed weight.

With reference to leaf size, it was varied from 2.3 x 0.7 to 4.3 x 2.5 cm with a mean of 3.09 x 1.31 cm. The leaf area was found increased in KAD2, MBT3, OTM3, TTG2 and TTG6, while, CYT1, CSM4, TAD, OJN3, OOS8 and RBU4 had reduced (2.3cm each) leaf area. The ratio of leaf length/width was found as characteristic feature to leaf shape and was 0.19 cm in round leaves to 7.66 cm in elongated leaves. The peduncle length was ranged from 0.6 cm for ecotype GTN1to 2.5 cm for ecotypes such as TGU3, SBG3 and RBU with an average of 1.59 cm. Figure 3 represents habit and diversity of leaves from *M. communis*var. *italica*.

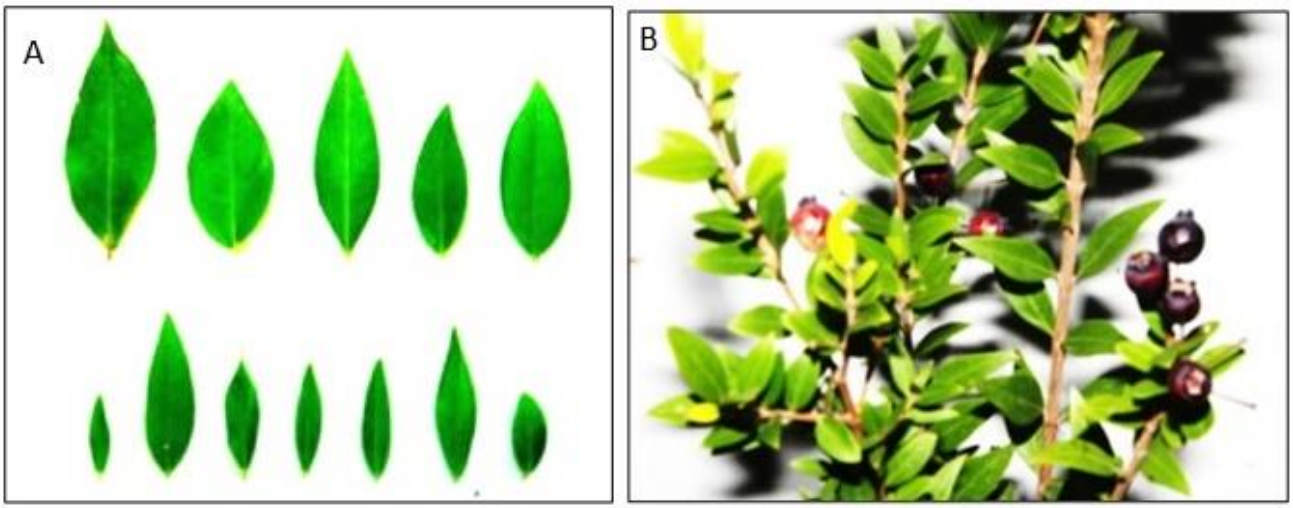

Figure 3. A - Habit and B - diversity of leaves from *M. communis* var. *italica*.

The calyx diameter was ranged between 0.26cm and 0.66cm with a mean of 0.47cm. The fruit number per shoot was found highly inconsistent and it is evident that the ecotype labeled KMD1 possessed on an average 1.1 fruit per shoot, whereas TNO4 retained 36 fruit per shoot (Table 1). The fruit length was between 1.4 and 0.6 cm and fruit width were ranged from 1.2 to 0.5 cm with an average of 0.79cm. The ecotypes such as OJN5, OOS12 and RBU4 possessed highest fruit length (1.4cm), whereas RBU2, OOS9, TST1 and SSO1 had lowest fruit length i.e., 0.6 to 0.7cm. The fruit width was ranged 0.5 to 1.2 cm with a mean of 0.79cm. The germplasm such as TNO1, TKN1, TBA2, CPU, CSM3, CSM5, MBT3, TMC2 and RBU5 possessed highest width that was 1.2 to 1.1 cm respectively. While some ecotypes such as SBG4, OJN3, OJN5, OSN1, OOS3 and OTM1 had lowest fruit width i.e., 0.5cm. The ratio of fruit length to fruit width, a characteristic that depended on berry shape, varied between spherical to pyriform and oblong. The fruit volume was ranged 0.22 to 0.97ml with the average value of 0.54ml.The highest fruit volume was observed in MBT3 (0.97ml), followed by MBT2 (0.96ml), CSM2 (0.94ml), RBU4 (0.93ml), CSM3 (0.92ml), RBU3 (0.91 ml) and CSM1 (0.90ml). The fruit weight was ranged between 0.17 to 0.88 g with an average of 1.39g. The highest fruit weight was noted in MBT3 with 0.88g, followed by MBT2 (0.87g), CMSM2 (0.84g), RBU3 (0.83g), CSM3, RBU4 (0.81g each), and CSM4, RBU2 (0.8g each). On the other hand, TTG3, OOS12 and KMD2 possessed the lowest fruit weight (Table 1). The heaviest fruit corresponded to the largest fruit volume.

The ratio of pulp was ranged from 0.14g to 0.15g with a mean of 0.33g. The ecotype SBG4 possessed highest pulp (0.87g), followed by MBT3 (0.76g) and MBT2 (0.75g), whereas lowest pulp yielding ecotypes were SBG3 (0.03g), TNO6 (0.04g), TTG3 (0.05g) and OOS9 (0.06g). The seed weight per fruit was ranged from 0.01g to 0.7g with a mean of 0.13g. The ecotypes labeled as TMC2 had highest seed weight (0.7g), followed

by TNO6, SBG3 (0.4g each) and RBU1 and RBU3 (0.21g each). While some ecotypes such as BKC1, CYT1, OOS13, OOS6 and TST1 had low seed weight (0.01-0.06g respectively).

A significant correlation was observed between fruit weight and fruit volume (Table 2). Large fruit had few small seeds. Shoot length was correlated to internodal number with more vigorous shoots, such as those of ecotypeslabeledOOS13 (30 cm) and MBT3 (29.96 cm). A negative correlation exited between internode number and leaf size, while correlation between internode length and leaf size was positive, revealing that ecotypes having a high number of short internodes had smaller leaves.

Table 2. Correlation coefficient values between biometric parameters.

	Fruit weight(g)	Pulp (g)	Seed weight (g)	Fruit volume (ml)	Fruit length (cm)	Fruit width (cm)	Fruit length/width
Fruit weight(g)	1						
Pulp (g)	1.000**	1					
Seed weight (g)	0.609**	0.602**	1				
Fruit volume (ml)	0.091	0.089	0.273**	1			
Fruit length (cm)	0.000	-0.002	0.210*	0.162	1		
Fruit width (cm)	0.054	0.053	0.099	0.008	0.380**	1	
Fruit length/width	-0.004	-0.005	0.042	0.147	0.401**	-0.607**	1

**. Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed).

4. Discussion

Dark myrtle is fairly distributed in the study area; however, fruit is ripened in different time period due to microclimatic flexibility. This study identified five main regions viz., Rabat (Balu), Cotton lower (Siah Mano), GujarTangi (Mian Banda Tall), Talash (Narowobu) and Macho with the abundance of its distribution in the form of forests. These localities are situated in both southern and northern aspects. The germplasm that grows in northern sides have greater opportunities to trap maximum sunlight and were highly palatable coupled with the yielding attributes. The local communities collect and procure fruits of the said species and sell out in the market for earning their livelihood. According to our survey, a metric ton of fruit is sold in the local market that is worth of Rs. 250000 in Pakistan rupees. This fruit is known as *Habb-ul-Aas* used in herbal medicine.

The biometric analysis of selected ecotypes revealed high variability for all studied characters, especially for characteristics useful in selecting cultivars for commercial production. The bushy plant along with good leaf area, and fruit characters such as volume, weight and pulp were the ideal characters to determine the myrtle cultivar. The present study suggests that MBT3 ecotype possessed fairly good characters such as high shoot length (29.96cm), leaf area (2.3cm), fruit volume (0.97ml), fruit weight (0.88g) and pulp (0.87g) and found the best suited for cultivar development.

With reference to various parameters, various ecotypes/germplasm had variability in morphometric features. The highest shoot length was recorded in OOS13 (30cm) and MBT3 (29.96cm). The largest internodes were recorded in BJT1 with 28 number, whereas TBA1 possessed highest length (1.8 cm). The leaf area was found expended in KAD2 and MBT3 (2.3 cm each). The peduncle length was prolonged in TGU3 (2.5 cm). The ecotype labeled TNO4 retained 36 fruit per shoot, while; OJN5, OOS12 and RBU4 possessed highest fruit length (1.4 cm). The highest fruit volume and fruit weight was recorded in MBT3 (0.97 ml and 0.88g). The ratio of pulp was higher in SBG4 (0.87g), followed by MBT3 (0.76g) and MBT2 (0.75g). The ecotypes labeled as TMC2 had highest seed weight (0.7g).

There was positive correlation observed in fruit weight and fruit volume (Table 2). Our results agree with those of Mulas and Cani 2008, who reported that that there is positive correlation between fruit volume and fruit weight. Large fruit possessed much of juicy pulp as well as less seeds, whereas those ecotypes which possessed small fruits had maximum number of seed with less flash. With reference to the distribution of myrtle ecotypes, our results disagree with the finding of Mulas and Cani 2008, who reported correlation amongst various parameters, such as fruit width and fruit length/width ratio. The aforementioned parameters were found to be negatively correlated that may be attributed to difference in agro-climatic conditions and altitudinal gradients. These were collected ecotypes ranging from 10 to 637 meter above sea

level, whereas our ecotypes were recorded from 848 to 1734 meter above sea level and located in all hilly areas.

5. Conclusions

The present study suggests that MBT3 ecotype possessed fairly good characters such as high shoot length, leaf area, fruit volume, fruit weight and pulp and found the best suited for cultivar development.

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