

Supplementary Table S1: Details of populations studied in the present study. Subdioecy is prevalent in 12 natural populations present in three geographically isolated valleys.

S. no.	Population	Valley	Population Code	Geographical correlates	Population	Gender of Collected Genotypes			Total
						Male	Female	Polygamomonoecious	
1.	Takmar	NUBRA	TK	34°22'.542"N 77°40'.041"E	Dioecious	3	1	-	4
2.	Shyok River Bed		SR	34°26'.937"N 77°43'.886"E	Dioecious	2	2	-	4
3.	Khalsar		KH	34°27'.642"N 77°42'.614"E	Subdioecious	-	4	1	5
4.	Khalsar		KS	34°29'.730"N 77°42'.713"E	Dioecious	2	2	-	4
5.	Khalsar		KR	34°30'.476"N 77°42'.414"E	Dioecious	3	3	-	6
6.	Sumur		SU1	34°36'.573"N 77°37'.065"E	Dioecious	4	3	-	7
7.	Sumur		SU2	34°36'.420"N 77°36'.703"E	Dioecious	3	3	-	6
8.	Sumur		SU3	34°32'.444"N 77°39'.408"E	Subdioecious	3	3	1	7
9.	Hundar		HS	34°34'.357"N 77°29'.702"E	Dioecious	4	3	-	7
10.	Diskit		DK	34°32'.656"N 77°34'.256"E	Subdioecious	3	3	1	7
11.	Basgo	SURU	BA	34°13'.113"N 77°16'.715"E	Subdioecious	1	1	3	5
12.	Saspool		SP	34°14'.768"N 77°09'.679"E	Subdioecious	1	3	3	7
13.	Ultopo		UT	34°15'.318"N 77°05'.589"E	Subdioecious	2	3	1	6
14.	Nimmu	INDUS	NI	34°11'.199"N 77°20'.537"E	Dioecious	3	3	-	6
15.	Upashi		UP1	33°50'.489"N 77°48'.266"E	Subdioecious	-	-	4	4
16.	Upashi		UP2	33°45'.914"N 77°45'.815"E	Dioecious	2	-	-	2
17.	Upashi		UP3	33°49'.434"N 77°49'.125"E	Dioecious	1	2	-	3
18.	Gya		GY	33°37'.301"N 77°45'.241"E	Subdioecious	-	-	1	1
19.	Lato		LT	33°41'.777"N 77°44'.498"E	Subdioecious	-	-	3	3
20.	Ranbirpura#		RP	34°00'.465"N 77°41'.514"E	Dioecious	9	10	-	19

S. no.	Population	Valley	Population Code	Geographical correlates	Population	Gender of Collected Genotypes			Total
						Male	Female	Polygamomonoecious	
21.	Shey#		S	34°05'.223"N 77°38'.289"E	Dioecious	7	7	-	14
22.	Sindhu Darshan #		SD	34°05'.269"N 77°36'.687"E	Dioecious	3	3	-	6
23.	Choglamsar* #		CV	34°05'.236"N 77°36'.090"E	Subioecious	7	7	4	18
24.	FRL#		FRL	34°08'.564"N 77°30'.450"E	Subioecious	6	6	5	17
25.	Water Park*#		WP	34°08'.115"N 77°30'.098"E	Subioecious	1	1	5	7

* Populations where floral biology studies were conducted to study the gender expression. # Populations sampled for isolation of gender linked SCAR marker. Finally isolated male specific SCAR maker was validated in all populations.

Supplementary Table S2: Primer combination used in AFLP. ✓ mark shows the particular combination of EcoRI+selective nucleotides and MseI+ selective nucleotides. ✓ (red) mark and green mark (✓) depicts the primer combination which generated female and male specific fragments across populations respectively. The sequence of primers used for preamplification (EcoRI and MseI specific) was adapted from Vos *et al.*, 1995.

MseI	EcoRI							
	E-ACG	E-ACT	E-AGC	E-AGG	E-AAC	E-AAG	E-ACA	E-ACC
1. M-CAA	✓	✓	✓	✓		✓		
2. M-GAC	✓	✓	✓	✓		✓		✓
3. M-CAG	✓	✓	✓	✓				✓
4. M-CAT	✓	✓ (red)	✓ (green)		✓			
5. M-CTA	✓	✓	✓	✓		✓	✓	✓
6. M-CTC	✓	✓	✓	✓			✓	
7. M-CTG	✓	✓	✓	✓ (red)	✓ (red)			✓
8. M-CTT	✓	✓	✓	✓	✓			✓
9. M-CAGT	✓	✓	✓	✓ (red)				
10. M-CTAG	✓	✓	✓	✓	✓			
11. M-CTGC	✓	✓	✓	✓				✓
12. M-CGAT	✓	✓	✓	✓		✓		✓
13. M-CACG	✓	✓ (red)	✓	✓		✓		

Supplementary Table S3: List of primer pairs designed from HRMSSR locus to test its gender specificity across populations. These primer pairs were also used in different combinations to characterize the locus in male and female plants.

Serial No.	Number indicates the Position/region amplified with the primer pair in HRMSR locus (5'-3')	Primer pair
1.	146-607	FP: AGATATAACCATGTGAATCG RP: ACATATAGAAGTTGGAAG
2.	954-1281	FP: CAGAGTCAATTCTAGCTCCT RP:CTCAAGTGCACTAGCCAT
3.	1318-1767	FP:ACGTTGAATTGTTCGCACA RP: AGGTTGGTGAGGAAACATCA
4.	2160-2323	FP:TGGAATTAAGCATCCCT RP:AGGTCCTTGTTTTATTCA
5.	2355-2847	FP:CATGGCTTGGTGCTCAAAGA RP:GAAGTTGCCAACTTGCTACA
6.	2967-3350	FP:TGACAACGAGCCAATTCTT GAATCATTAAATTGGTCGGA
7.	2967-4070 (HRSS1-HRML2)	FP:TGACAACGAGCCAATTCTT RP:CACTCGGCGCTCTAGGATAG
8.	3742-4070 (HRML1 and HRML2)	FP:GGTTCATTTCTGTTACTCTGA RP:CACTCGGCGCTCTAGGATAG
9.	3742-4419 (HRML1 and HRML3)	FP:GGTTCATTTCTGTTACTCTGA GAGCTTGTAGAATACTGTGG
10.	4518-4998	FP:AATCCCATAAACTGCCTCGA RP:ACATTCGCTTAAGAATTGGCA
11.	4621-5335	FP:GCTCACTAGCAAAGCAACGT RP:ATCTTGTTGGACTTCTTGCTAGAG
12.	5452-5995	FP:TAACCTATGAGCTTAGGCCG RP:ATGCCTTTTGTTGAGGTTG
13.	6229-6613	FP:GCTGAGGCATTAATTAGCCC RP:ATGGGTAGCGATATGGTCTCAC

Supplementary Table S4: Summary of homology search of RDA based sequences at NCBI and Repbase databases

Sl. No	Primer Name	Homology at NCBI database					Homology at Repbase database				
		Sequence	Query coverage (%)	E-Value	Identity (%)	Accession no.	Sequence	Dir	Sim	Pos/Mm:Ts	Score
1.	RDAP-27	<i>Hippophae rhamnoides</i> clone pLBSSR2123 microsatellite sequence	42	3 ^{e-93}	98	GQ241403.1	Satellite; Simple Repeat; Sat1_Mad, from <i>Malus domestica</i> (apple),(Rosaceae)	c	0.9344	1.5000	966
2	RDAP-33	<i>Vigna angularis</i> var. <i>angularis</i> DNA, scaffold: Scaffold_1655, complete sequence, cultivar: Shumari	4	4 ^{e-05}	86	AP016699.1	Gypsy; LTR Retrotransposon; Transposable Element; Gypsy2_Pru_; from peach (<i>Prunus persica</i> , Rosaceae)	c	0.6667	1.8873	490
3	RDAP-41	<i>Hippophae rhamnoides</i> clone pLBSSR2123 microsatellite sequence	96	3 ^{e-175}	95	GQ241403.1	EnSpm/CACTA; DNA transposon; Transposable Element; CACTA; Caspar; from <i>Triticum monococcum</i> (Poaceae)	d	0.6931	1.4211	269
4	RDAP-47	<i>Hippophae rhamnoides</i> clone PLBSSR2094 microsatellite sequence	59	7 ^{e-51}	89	GQ241412.1	Internal region of LTR retroposon, SHACOP_MT, from <i>Medicago truncatula</i> (Fabaceae)	d	0.7692	1.2632	554

Dir- stands for direction: complementary (c) or direct (d); Pos is roughly the ratio of pairs of bases that produce positive scores in the alignment matrix to the alignment length; Mm:Ts is a ratio of mismatches to transitions in nucleotide alignment

Supplementary Table S5: Result of sequencing, BLASTn and Primers sequence (nucleotide sequence in red colour) designed for the conversion of sequences obtained by AFLPs into gender-linked SCAR markers.

Sequence from Selective Primer combination	Sequence (5'-3')	Primer (5'-3')	Blast result	e-Value	Anealing Temperatur efor SCAR	Specificity0f SCAR primers
E-ACT + M-CAT	GACTGCGTACCAATTCACTG ATGCTACTCAACTGG GATT TGAGCCGATAGACTATGAGGATCTCTCAGAA GCCCTAACTCATCTAAGGCATACCACTAGTGTAGT AGCATACCAGGAAGCGTTTGAAAATTGTCACAG AGTGGACGGTTTACCAAAATAATTCCTAATAGGGT GATTCATAGTT GGACTGAGGGATAAAATATGCC TT GATGTTACTCAGGACTCTC	FP: ATGCTACTCAACTGGGATT TG RP: GGCATATTTTTATCCCTCAG TCC	<i>Populus trichocarpa</i> clone POP029-P20, complete sequence and <i>Vitis vinifera</i> contig VV78X033551.38, whole genome shotgun sequence	2e ⁻⁰⁸	50°C/52°C	None
E-AGG + M-CAGT	GACTGCGTACCAATTCAGGCA CAAGATAATGCACA TCATATTC CACACAAGTATATATTAGTCAAGTCAG ACCCTACTTGAAAATAGTAAGGCAAGAGACACACA AGCATGTAGCAGAGGCACACATAATGTCATAATGA TACAAGCAGAAGTCAAGCTAGCAAAAAACAGAAT AAAATAGATTGAGTTAG GC AAAG AAGAAGCTCGA GACT TACTGTTACTCAGGACTCATC	FP: CAAGATAATGCACATCATA TTC RP: GTCTCGAGCTTCTTCTTTGC C	No significant match		50°C/52°C	None
	GATGAGTCCTGAGTAACAGTAAGTCTCGAGCTTCT TCTT TGCCTAACTCAATCTATTTTG TTCTGTTTTTTG CTAGCTTGACTTCTGCTTGTATCATTATGACATTAT GTGTGCCTCTGCTACATGCTTGTGTGTCTCTTGCC TACTATTTTCAAGTAGGGTTTGACTTGACTAATATA TACTTGT GTGGAATATGATGTGCATTATC TTGTGCC TGAATTGGTACGCAGTC	FP: TGCCTAACTCAATCTATTTT GT RP: GATAATGCACATCATATTC CAC	No significant match		50°C/52°C	None
	GATGAGTCCTGAGTAACAGTGCTAGCTGTTTATAA TCATGATATAATA ATAACGTTGTTATGCTTGCGAA ATATTAAGTTACCGCTCGGAATTATCTATTTTTCG GCAAATTCGAACGGCCGCGATCCCTCTCCTTGATC CATTCCACCGTAGCTATG CGTTTTCTATTTTTTTAT TTCTTTTCTACTTCCCCCTGATTGGTACGCAGTC	FP: ATAACGTTGTTATGCTTGC GAA RP: CATAGCTACGGTGGAAATGG	<i>Hippophae rhamnoides</i> clone pLBSSR2123 microsatellite sequence	1e ⁻³⁵	50°C/52°C	None

E-ACC + M-CTG	GACTGCGTACCAATTCACCCAGAGACGAGGGCGAA GATATTGAGAAAATGCGCGAACGCGCCAGTAAGA ATCACACCTTTGTGTATGTCAAAATTCAGGCATAC CAGTGAAAAGTTAGTTACAAGGGCAAAAAGCAGAA GACTGTGACAGACTTGAAGAACATGAGCTTGACAT TGCCCACTTACGAGTATCCCAATTCCTACTCGACTT GGTTGGATCTATTGATGGCAGTTACTCAGGACTCA TC	FP: AGACGAGGCGAAGATATTG AGA RP: ATCAATAGATCCAACCAAG TCG	No significant match	5e ⁻⁰⁴	50°C/52°C	None
	GATGAGTCCTGAGTAACTGATTC AAGTGCTTCACA TCACATGACATCAAATCCTTCAAATCTTGTAATCAA AACTAAATATGAAGGCTTTTATTAATATTTTGATT TTCAAGATCTTGAAAAGATTCTTCTTGTTCTTTA GAGTCCTTTTTGGATTAGGTTTTCAAATCTGATT TTTTTAGATTGTCACTAACAAACCGGTTACTTCTTG AGATTAGGTGAATTGGTACGCAGTC	FP: TCAAGTGCTTCACATCACA T RP: AGAAGTAACCGGTTTGTTA G	No significant match	-	50°C/52°C	None
	GATGAGTCCTGAGTAACTGATGGAAGTTTTATGGA TCAAATTTAGGCTGAGGAGAAGCTAAGGGTGATGG TAAAGACTAATTCAAATGTGGAACAAAACCTTTGC CAAAATGATACCAGCAAGCAGAGACAATATGCCCT AACTTTCTACATAACTGGCACTATGGACGATTGTGT CTTCTTCTACCACGACCTCTTCCAAAGTTATTATAA GGATCAAACGGTGAATTGGTACGCAGT	FP: GAAGTTTTATGGATCAAAT TTAGG RP: TGATCCTTATAATAACTTTG GAAGG	No significant match	-	50°C/52°C	None
E-AGC + M-CAT (HRML)	AGAATAGAATTGAGAGAGAAAAGAGAAAAGAGAGT TAGGGAGGCAGAGGGATAATTGGTTTCATTTCTGT TACTCTGATGCCTAACTATTTGCTCAATGGTCCCC TCCTACGCCGTTCTGTGTTATCATGTGCTCAGTTG CACCCACTGCGTGCCGCATGTGTGTTGGTCTGTTGCG TGCGCGGTTGTGCGAGTAGAATCTTCATGTAACAT TTCTCCCCCTCTTTCAAATCCTTGCCACAAGGAT GAAGTCAGGATAGGACTTGGAGAAGCGCCATTCAT TCTCCAACTAGCTGCCTCGGTTGGAGCCCCCTACCC ATTTGACCAGAGTTTCAAATTTGGTTGATATCTAT CCTTCTGAATCACTCGGCGCTTAGGATAGCTTCAG GCTGTTTATGATCGTTGAGTGTCTGAGATTGAAGT TAGTTGGGAGATGGTAGGGTAATGGTCTTATGT GCTTCTTCAAC	FP:GGTTCATTTCTGTTACTC TGA RP: CTATCCTAGAGCGCCGAGT G	<i>Populus trichocarpa</i> clone POP029-P20, complete sequence Or <i>Vitis vinifera</i> contigs..genome shotgun sequenece	1e ⁻²⁸	50°C/52°C	Male

E-ACT + M-CACG (AFLP-32)	AGCAATTTAGGGAGCGACACATGATAGGGGAGAC CGATTAGAGGCAATGCTCGCTGAGTTAGTGACATG TGGCATGTTGACCAATTTTTGCACTTGGATTGAACG TTATGCTAATACGTGACACACGTTTCATCCTCCTCA CACGCGGGGTGATATGATTCTCTCTTTGTGCTTGTG TGGTTTGACTAGTCAACTCATTTGATTGTGTGACAT GACA	FP:AATTTAGGGAGCGACAC ATGATAG RP: GTCATGTCACACAATCAAA TGAGTTG	No Match	-	64°C	size variation in some populations while for some populations it gave two bands in male for others in female
E-AGG + M-CTG (AFLP-15)	CGACGGCCCGGGCTGGTAAATTTAATTTAATTATAT AATATGTGCATATTTTTAAGCTTCAAGCTTAATTA CTTATTAGAATGTGGTTGGGAATAAAGTTATGATT TTTGAAGTAAATAGTTGCACTTAGTATT CACCTAAG GGTTGAAGTATCCAA TTTTAAAAGGAGTACTACAT AAACAATAGAATAATATTGTTTGAATATGGTTTTG AATAAAATTATACTTTTTGAAGTAAATTTTGTATT AATATGCACCGAAGAGTATCTTATGAATAATATTG AGGGTCAATAATAGTAGTGAAAGATTTAGCATCTG AATTCAGGTGGGACTAGAGTTGTCAATGAGGCCAA GTCGGCT CAATACGGTATGGTCTAGT CAGTTAATT AGTATGTCATGTCTATATTTTTAGCCTGTGGCTTTT ATCGTATCATGCTTGGTTCGTTAAGTTGTGCTTAAT AGGACTGTATAACTTGTGGCCCATTTAGTCCATTAT TTGAAAAAATACCAGCCCCGGCCGTCGACCACGCG TGCCCTATAGTA	FP: CACCTAAGGGTTGAAGTAT CCAA RP: CAATACGGTATGGTCTAGT	<i>Vicia faba</i> var. <i>minor</i> aap1 gene, promoter region.	1e ⁻⁰⁹	66°C	AFLP based primers amplified female specific amplicon. However, when converted into SCAR marker, primer turned out as male specific marker for some populations