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RESEARCH ARTICLE

GENETIC DIVERSITY AMONG FOUR *MOMORDICA* SPECIES USING RAPD, SSR AND ISSR MARKERS

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Abstract

Background: Genus*Momordica*,iswidely distributed overtropicandsub-tropic region. Besidesthecultivatedbitter gourd(*M.charantia*L.var*charantia*),manyotherspeciesofthegenusoccurringinthewildstatehavebeenfound inIndiaandneighboring countries.Amongthem,monoecious,*M.charantia*and*M.balsamina*anddioecious,*M. dioica*and*M.cochinchinensis*exhibitdivergenceinmorphologicalcharacterslikegrowthhabit,maturity, andfruit shapeandsize,sexexpression,leaf,rootandseedcharacters.

Methods: We studied the twenty-fouraccessionsrepresentingfour*Momordica*speciesincludingtwogynoecious linesof *M.charantia*namelyGy-323,Gy-333 by morphological and molecular analysis through RAPD, SSR and ISSR markers.

Results: The analysis of study based on dendrogram obtainedfromthedistancematrixfromthemeanvalueofthe 15quantitativetraitsforthegenotypesgroupedalltheaccessionsintotwomajorclusters. Onecluster separatestwodistinctgroupseachaccessionsof*M.charantia*and*M.cochinchinensis*and*M.balsamina*formed distinctgroupwithina major clusterwhilethesecond major clusterconsistedofallaccession of*M.dioica*.For moleculardiversityanalysis, 101 primersincluding50RAPD, 16SSR and35ISSRprimersproducedatotal of600 scorable amplicons across fourspecies, ofwhich586(97.08%) werepolymorphic. Seventeen markersdifferentiatingmonoecious anddioeciousand85-amplicons specifictooneofthefour*Momordica*specieswere identified.TheUPGMAdendrogramobtainedfromJaccard'ssimilaritycoefficient(averagesimilarityof0.38) showedtwomajorclustersclearlydistinguishing monoeciousanddiociousspecieswithhighbootstrapvalue(100) betweennodes.

Conclusions: Inthepresentstudy,morphological diversity

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basedonquantitativetraitsandmolecular diversityshowedsome correspondenceintheclusteringpattern ofaccessionsrepresentingaspecies.Molecularmarkersweremoreefficientindifferentiatingtheaccessionsatandwithinspecies levelthan morphologicalmarkers.

Introduction

Bitter gourd(syn. bitter melon, Momordica*charantia L.*), aneconomicallyimportant memberofthegenus*Momordica*(Family:Cucurbitaceae)iswidelycultivatedinIndia,China,Malaysia,AfricaandSouthAmerica(Raj et.al.1993;Singh 1990)foritsimmaturefruitsasvegetable. Bittergourdisalsousedasatraditionalmedicinefor diabetes in India,ChinaandCentralAmerica (Groveretal.2002;Yehetal.2003),vaccine (Duttaetal.1981)and otherhealthrelatedailmentsduetoitshealthpromotingsubstancessuchascharantin(Yehetal.2003).Thegenus *Momordica*comprising of59speciesiswidelydistributedallovertropicandsub-tropicregionbutthediversity of thespecies ismoreintropicalAfricaincludingseveralspeciesofeconomical andmedicinalimportancepossessing differentsexformanddifferentbasicchromosome number.Aboutathirdofthespeciesaremonoecious and remainingdioecious(Schaefer,2010).Theincompatibility among2n=2x=22,2n=2x=28and2n=4x=56speciesin thisgenusisindicatedbythenegativeresultsofcrossingsbetween *M.charantia*L.and*M.muricata*L.and between *M.dioica*Roxb.*M.cochinchinensis*Spreng.(TrivediandRoy1972).Besidesthecultivatedbittergourd(*M. charantia*L.var*charantia*),severalspeciesarepresentinIndia,SriLanka,Bangladesh, Myanmar,Malay,etc. (Hooker1879)occurringinthewild stateandaregatheredbytribalcommunitiesasvegetables.Sevenspeciesoccurin Indiaandamongthem*M. charantia*and*M.balsamina*aremonoeciouswhile*M.dioica*,and*M.cochinchinensis*aredioeciousspeciesandarepropagated vegetativelythroughtuberousroot(Rashid1993).Thesespeciesexhibit morphologicaldivergenceincharacterslikegrowthhabit,maturity,fruitshapeandsize,sexexpression,leaf,root andseedcharacters(RobinsonandDecker-Walters 1999).Thefruitsarebitterintasteandaredesirablefor consumption.Thus,*M.charantia*isperhapsistheonly speciesinwhichbitternesshasbeen consideredasatraitfor selectionduringdomestication. Besidesmanyculinary usesinsouth,southeastandeastAsia,thegenusisalso cultivatedforitsuseasfolkmedicines.

*M.charantia*provides forrelativelybroad phenotypicvariation (Kundu 2012, Iqbal 2016), whichproduceslargefusiformfruits, while*M.dioica*isanimportantvegetableintheIndiansub-continent. Ithasmanyadvantages,likehigh marketprice,goodnutritionalvalueandlonger shelf life(Rasul2003, Singh 2013).Sweetgourd(*M.cochinchinensis*) occursinpeninsular region,humidtropicalforestsandalsoinnorth-eastern regionandbalsampear*M.balsamina*occursin north-western,Indo-Gangeticplainsadaptedtodrysandysoils.Sweetgourd,teaslegourdand balsampear beingminorvegetables.

Application ofmolecularmarkersastoolforidentificationandcharacterization ofgermplasmfortheir efficientmanagementanduseinplantbreedinghasgainedimportance(Karp&Edwards1997).Amongthe differenttypesofmolecularmarkers,randomamplifiedpolymorphicDNA(RAPD)(Williamsetal.,1990)is usefulfortheassessmentofgeneticdiversity(Ulanowskyetal.2001;Baranskietal.2001;Pradeepkumaretal. 2003;Caietal.2007;Tiwarietal.2009;).Thetechniqueisideallysuitedforfingerprintingapplicationsbecauseitis fast(Gwanamaetal.2000),requireslittlematerial(Loweetal.1996)andtechnicallyeasy(FenwickandWard 2001).Thewideavailabilityofcommercialprimersmakesthistechniqueswidespread (Gilliesetal. 1997), inexpensiveandyieldslargenumbersofmarkers(MartinandBermejo2000).

Recently,someunderstanding ofthegeneticvariationshasbeenachievedusing transcriptome analysis of within *M.charantia*species (Shukla et al. 2015) and molecularmarkerslike RAPD (Deyetal.2006; Beheraetal.2008;Bharthietal.2012), Inter SimpleSequenceRepeat (ISSR) (Singhetal.2007; Bharthietal.2012),AFLP (Gaikwadetal.2008)andSimpleSequenceRepeat (SSR)(Jietal.2012)in*M.charantia*andinrelated species(*M. dioica*)usingRAPD(Rasul etal.2007)andSSRmarkers(Jietal.2012).TransferabilityofSSRmarkershasalsobeenreportedinrelated

species(Jietal.2012).Although,mostofthesestudieshavefocusedontheefficiencyofdifferentmolecular markersforassessingthediversitywithinonespecies,noneaimedatassessingthediversity amongsomerelated *Momordica*species.Thepresenteffortsaimtogetacomparativeaccountofmorphological andgeneticdiversity acrossthegenusandunambiguously identify differentspeciesof*Momordica*usingmolecularandmorphological markers.

MaterialsandMethods

Plantmaterials

Twentyfouraccessions offour*Momordica*speciescollectedfromdifferentagro-climaticzonesofIndiawere selectedbasedonmorphological diversityandgeographicaldistributionandwereevaluatedattheResearchFarm, IndianInstituteofVegetableResearch,Varanasi,(U.P.)duringrainy-winter seasonof2014. Thelistofaccessions includedinthestudyisdetailedinTable 1.

Table 1: SalientFeaturesofusedMomordicagenotypes.

Species	Source	SalientFeatures
Spinegourd(*Momordica charantia*)		Monoecious,trailingvine withthinstemsandtendrils,Theleavesare heart-shaped,5-10cmindiameter,cutinto5-7lobes,Flowersare bornesinglyinthe leafaxils,fruitsaresmallto verylarge,splitopen,revealingorange fleshandbrightredplacentato whichtheseedsareattached,Seedsaretanandoval,witharoughetched surface.
GY-323	IIVR	Gynoeciousplant,Largevines,largesizeleaveswithheartshape,
GY333	IIVR	Gynoeciousplant,mediumvines,morebranching,profusebearing,greenfruis
DRAR-1	TNAU	Monoecious,large vines,mediumleaves,lessbranching,lightgreenfruits
VRBT-1	ANGARU	Monoecious,morebranching,mediumsizeleaves,continuousfruitingfor longerperiod,greenfruits
MC-84	TNAU	Monoecious,mediumsizeleaves,lightgreenfruits,mediumsize fruits
DVBTG-7	IIVR	Monoecious,mediumsizeleaves,morebranchinggreenfruits,mediumsize fruits,
DVBTG-5	IIVR	Monoecious,mediumsizeleaves,morebranchinggreenfruits,mediumsize fruits,
PDM	IARI	Monoecious,mediumsizeleaves,morebranchinggreenfruits,smallsize fruits,
Sweetgourd(*Momordica cochinchinensis*)		Dioecious,rootsdevelopsbiggertubers,leavesarebigger,flowersarelarge, yellow,3 smalldeepblackorbluecirculardotsatthe baseofpetal,Flowerslarge oblongfruits,fruitsarelightgreentoyellowin colour.
RCSG-1	ICARRes. Complex Shilong	Dioecious,vigorousplant,lightgreenfruits,morefruiting
RCSG-7	ICARRes. Complex Shilong	Dioecious,mediumplant,lightyellowfruits,mediumfruiting
RCSG-5	ICARRes. Complex Shilong	Dioecious,vigorousplant,morespinyfruits,morefruiting,yellowfruits
RCSG-36	ICARRes. Complex Shilong	Dioecious,vigorousplant,toughrind,less fruitinglightgreenfruiting
DRMC-22	Kalayani (W.B.)	Dioecious,mediumplant,toughrind,mediumfruiting,greencolourfruits
DRMC-25	Kalayani W.B.)	Dioecious,vigorousplant,softrind,moreseed,mediumfruiting,lightyellow greenfruits
Spinegourd*(Momordica dioica*)		Dioecious,rootsdevelopssmalltubers,smallleaves,flowersaresmallyellow colourandopenintheevening,nocirculardotsatthebaseofpetal,roundto ovalfruits, fruitsaredarkgreentoyellowincolour
VRMD-1	Varanasi	Dioecious,mediumviny,morefruiting
RSR/DR-1	Mirzapur	Dioecious,longviny,mediumfruiting
RSR/DR-2	Mirzapur	Dioecious,mediumviny,morefruiting
RSR/DR-3	Sonbhadra	Dioecious,mediumviny,less fruiting

VRMD-20-3	Satana	Dioecious,smallviny, mediumfruiting
VRMD-22-5	Satana	Dioecious,mediumviny,less fruiting
VRMD-4	Patana	Dioecious,smallviny,morefruiting
Balsampear*(Momordica balsamina*)		Monoecious,trailingvine withverythinstemsandtendrils,Theleavesare heart-shapedand3-6cmindiameter,cutinto3-4lobes,Flowersareborne singlyintheleafaxils,fruitsaresmallhavingridgesthroughout surfaceandintheendelongationtake place,splitopen,revealingorange fleshandbrightredplacentatowhichthe seedsareattached,Seedsare small,lightyellowandoval.
DRBS-1	Ranchi	Monoecious,thinandmediumspreadvines,boldsmallfruits withprominent distalend.
DRBS-2	ANGARU	Monoecious,thinandvigorousvines,mediumprominentdistalend,verysmall fruits
Sweetgourd (*Momordica cochinchinensis*)		
DRMC11	Bhagalpur (Bihar)	Dioecious,Dioecious,vigorousplant,softrind,moreseed,mediumfruiting, lightyellowgreenfruits

Fieldevaluationanddatacollection

Forfieldevaluation,theaccessionswereplantedfollowingtherecommended fertilizerdoseandculturalpracticestoraiseagood crop.Fiveplantswererandomly chosenwithineachaccessionfordatarecording,whichwasdoneforfifteen quantitativetraitsi.e.daysoffloweranthesis,ediblematuration,branchesperplant, plantheight,fruit diameter, fruitlength,fruit/plant,fruitweight,seed/fruit,yield/plant,petiolelength pediclelength,nodetofirstmaleflower, nodetofirstfemale floweremergenceandinter-nodallength.

ExtractionofgenomicDNAandquantification

TotalgenomicDNAwasextractedfrom thefreshyoung leavescollectedfrom fieldintheweehoursoftheday followingthe proceduredescribedbyDoyleandDoyle(1990)andmodificationsofMaguireetal. (1994).TheDNA solutionwaspurifiedandquantityoftheDNAwasestimatedbyrecordingODofspectrophotometer(GeneSpace-III,Hitachi,Japan)at260nm,andqualitywascheckedon0.8%agarosegel.

Polymerasechainreaction(PCR)assay andelectrophoresis

Outof120randomdecamerprimersfromOperonTechnologies, Inc.,AlmedaUSA,atotalof50primerswere selectedforRAPDanalysis.Thirty-fivecommerciallyavailableuniversalISSRprimersofUBCseriesand16SSR primers reportedinanearlierstudy(Wangetal.2010)wereusedinthePCR assay.PCRreactionswereperformed in96-wellplatesusingBio-radthermo-cyclersystemwiththesuitableamplification programusingatotalof50 randomdecamerprimers(OperonTechnologies,USA).Each25µlreactionmixturecontainedabout50ngof genomicDNA,0.2µMprimer,100µMeachofdATP,dGTP,dCTPanddTTP,25mMMgCl2and0.5Uof*Taq*DNApolymerase alongwithsuitable10xbuffer(10mMTris-Cl,pH8.3,50mMKCl,0.001%gelatin).The reactionwascarriedoutat 94ºC for4minaspre-denaturationstep,thenthe reaction wascycled40timesat94ºCfor 1min,33ºCfor1minandextensionat72ºCfor1min.Additionally,afinalcycleallowedextensionat72ºCfor10 min. ForISSRandSSRassay,annealingtemperaturewasstandardizedbeforeperformingtheassayoncompleteset ofaccessions.Carewastaken toensurethatasetofallaccession tobecomparedwereprocessedinthesame machine.

ThePCRamplifiedproductsfromRAPDandISSRassaywereloadedin1.5%agarosegelandseparated byelectrophoresis with1XTAEbufferat65Vfor1hrand30minutes.Thebandsizeswereestimatedby comparingwith bandsof1KbDNAladder (MBIFermentas),whichwas runalongwith the amplifiedproductsina separatelaneonthesamegel.ForproductsfromSSRassay,thefragmentswereelectrophoresed through2.5% Metaphoragarose(FMCBioproducts,USA)gelataconstantvoltageof90volts for5hours.Fragmentlengths were determinedwiththehelpof50bpladder(MBIFermentas,Germany)loadedseparately inthegelalongwiththe samples.Thegelswerestainedwithethidiumbromide(0.5mg/ml)andvisualizedinagel-documentedsystem (Alfa-Imager2200,Alfa-InnotechCorporation,California).ThePCRamplificationwasrepeatedtwotimesto ensurethattheamplificationobtainedwiththeprimersisconsistentandreproducible.

Dataanalysis

Morphologicalanalysis

Themean valueofthe datawasconsideredforcalculatingthegeneticdistanceamongthe accessions. DISTmodule ofNTSYS-pcwasusedtoachievethesame.TheNeiandLigeneticdistancematrixwas usedasinput file forgeneratingUnweighted Pair Group Method with Arithmetic Mean (UPGMA)baseddendrogramusingSAHNclustermoduleofNTSYS-pc.

Molecularanalysis

PCRamplifiedfragmentsofthe 24accessionwere scoredas present(1)orabsent(0) ofbands. Onlyclear,unambiguous ampliconsrangingfrom300bpto3000bpwerescored.Uniquepresenceorabsenceof singleorgroupofbandswasusedforidentificationofspecies.Resolvingpower (Rp)andpolymorphicinformation content(PIC)ofprimerswerecalculatedtotesttheefficiencyoftheseparameters inidentifying primersthatcould bestdistinguish thecultivars.Resolving powerisrepresentedasRp=ΣIb,whereIb=1-[2x(0.5-p)],pbeingthe proportionofcultivars containingthebands(PrevostandWilkinson 1999).PIC=1-(p2-q2),where p2isproportionof accessionshavinganampliconandq2isproportionofaccessionsnothavingtheamplicon(Rainaetal.2001).PIC valueofa primerwascalculatedbyaveragingthePICvaluesofallpolymorphicfragmentsgeneratedbytheprimer.

Thegeneticassociations betweenaccessionswereevaluatedbycalculatingthemodifiedNeiandLi's coefficientNL=1-[2N11/(2N11+N10+N01)](NeiandLi,1979)forpairwisecomparisonsbasedontheproportion of sharedbandsproducedbytheprimers,where,N11isthenumberofbands/allelespresentinbothindividuals,N10is thenumberofbands/alleles presentonlyinindividualAbutabsent inBandN01isthenumber ofbands/alleles presentonlyinindividualBbutabsentinA. Binarydatabasedonpresence(1)orabsence(0)ofbandswas analyzedbypair-wisecomparison usingJaccard'scoefficient(Jaccard1908). Thesimilaritymatrixthusobtained wassubjectedtoclusteranalysisbyUPGMAanddendrogramwasgeneratedtostudytherelatednessofthe cultivars.Therobustnessofthenodesofthedendrogramwastestedbybootstrapanalysisusing1000resamplings. Theseanalyseswerecarriedoutusing Freetreesoftware(Pavliceketal.1999).UPGMAdendrogramwasdrawn usingTreeview(Page1996).

Results

Morphologicalevaluationbasedonquantitativetraits

Themeanvalueof15quantitative traitsrecordedfor24genotypes representingfourdifferentspeciesofgenus *Momordica*(Supplementary Table S1)and highmorphologicaldiversityamongtheseaccessionsisnot surprising.Thedaysofflower anthesisandediblematurityismaximum(79.5and101.2)inDRMC-25andminimum (32.5and40.5respectively) is recordedinGy-333.Inaddition,thefruitlengthisrecordedhighest(18.98)inDVBTG-5,whereas,itisminimum (4.15)inVRMD-20-3. ThegenotypeDRMC-22showmaximumfruitweightandseedperfruit(126.35and24, respectively),whileitisminimuminVRMD-22-5 andDRBS-1(10.87and9)andgenotypeRSR/DR-3 showhigh seednumberofper fruit.Themaximumyieldperplantisrecordedin RCSG-1(2.255kg)andminimuminVRMD-1 (0.365kgs).ThegenotypeVRMD-20-3showhighest fruitsperplant(41.25)andisminimum(10.25)inDRBS-1.

Clusteranalysisbasedonquantitativetraits

Dendrogramobtainedfromclusteranalysisofthedistancematrixfromthemeanvalueofthe15quantitativetraits forthe24genotypes (Fig. 1)groupalltheaccessions intotwomajorclustersandseparatesatadistanceof29.00. Perusalofthedendrogramrevealstwomajorclusters.Alltheaccessionsof*M.charantia*(Gy-323,Gy-333,DRAR1, MC84,PDM,VRBT1,DVBTG7andDVBTG5);*M.cochinchinensis*(VRMD1,VRMD203,RSRDR3,RSRDR2, RSRDR1, VRMD225 andVRMD4) and*M.balsamina*(DRBS1andDRBS2)constituted distinctgroupswithinthe majorcluster.The secondmajorclusterconsistedofall accessionof*M.dioica*.However,RCSG-7wasdistinctfrom othersix genotypesand formeda distinctsub-cluster.

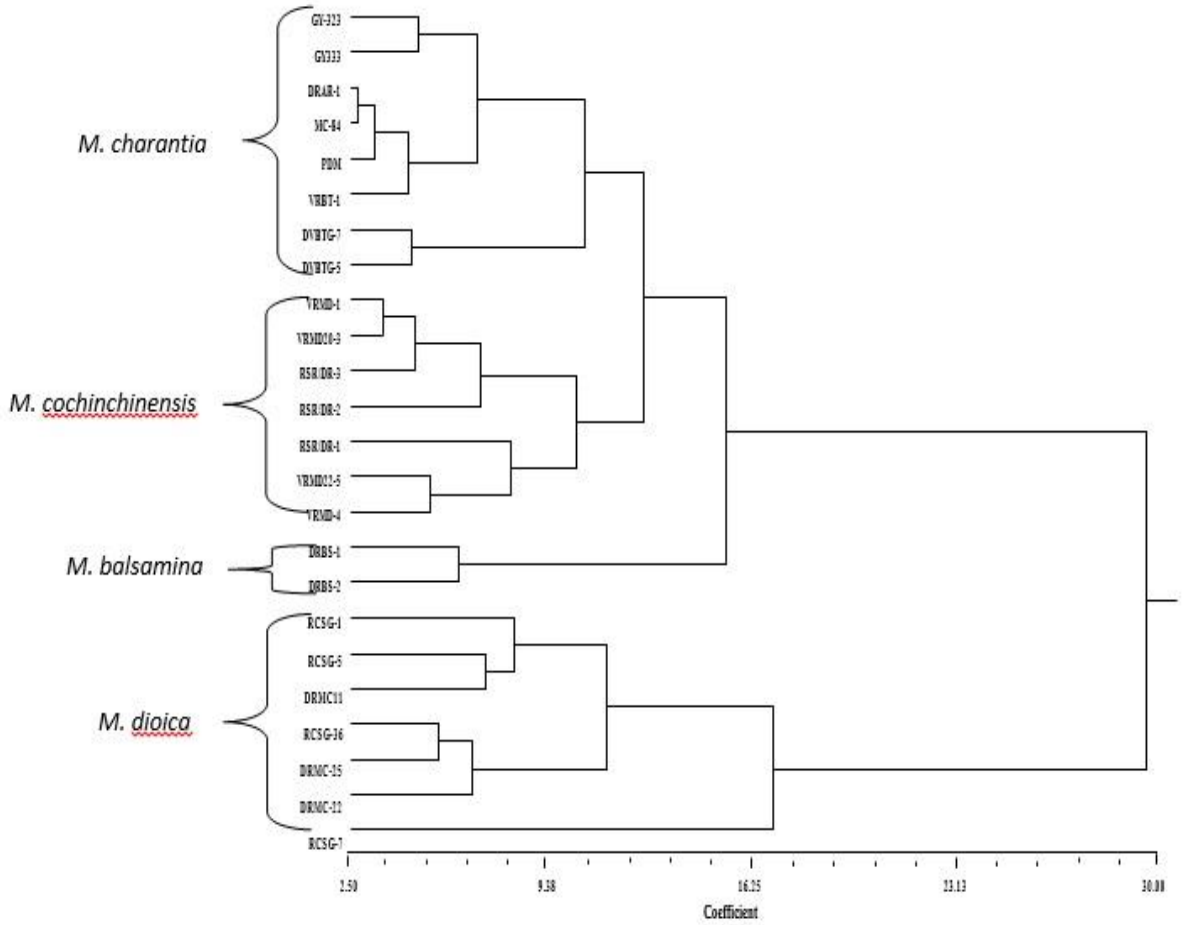

Figure 1: UPGMA dendrogramof Momordicaaccessionsgeneratedfrommeanvaluesof15quantitativetraitsused forclusteranalysisbasedonNei and Ligeneticdistance matrix.

Molecularvariationandspeciesrelationships

ThesequenceoftheRAPD,ISSRandSSRprimersusedforthemoleculargeneticfingerprinting ofthefour *Momordica*speciesandthebasicbandsproducedbyeachprimer,numberofpolymorphic bandsandpercentage of polymorphismproducedbyeachprimerarepresentedinsupplementary Table S2, S3and S4,respectively.Thebasicbandstatisticsof obtainedfrom thevarioussetofprimersindifferent combinationisalsogiven intable 2.Theprobabilityofchance identityoftwoaccessionswas 5.66x10-247indicatingthehighefficiencyofmarkersindifferentiatingtheaccessions.

Geneticdiversitywithinandamongspeciesassessedusing Nei'sgeneticdiversityandShannoninformationindexis givenintable3andsupplementary Table S5. Thishighlevelofpolymorphismcaneasilybeattributedtotheaccessionsrepresenting diversespeciesinthestudy.Theresultsarenotincongruencewithearlierstudies(Deyetal.2006andBeheraetal. 2008)duetonon-inclusion ofaccessionsrepresenting suchdiversespecies.Rasuletal.(2007)includedone accessionof*M.dioica*asanout-groupto studydiversityin*M.charantia*usingRAPDmarkers.

Table2: AnalysisofbandingpatterngeneratedbytheRAPD,SSRandISSRmarkerandcomparativeanalysisof differentmarkersystems.

Component	RAPD	SSR	ISSR	RAPD +SSR	RAPD +ISSR	SSR+ ISSR	RAPD+ SR+ISSR
No.ofprimers	50	16	35	66	85	51	101
No.ofmarkers	264	63	273	327	537	336	600
No.ofmarkersperprimer	5.28	3.93	7.8	9.21	13.08	11.73	16.49
Polymorphicmarkers	255	61	270	316	525	331	586
Polymorphicmarkersper primer	5.10	3.81	7.71	4.80	6.18	6.50	5.80
Polymorphism(%)	96.6	95.62	99.03	96.11	97.81	97.32	97.08
Assayefficiencyindex	5.1	3.81	7.71	8.91	12.81	11.52	16.62
Markerindex	72.93	75.73	78.70	148.66	151.63	154.43	227.36
Averageresolvingpower	3.244	1.933	4.957	5.177	8.201	6.89	10.134
AveragePIC	0.755	0.792	0.794	1.547	1.549	1.586	2.341
CorrelationbetweenRP and PIC	0.053	0.521	0.083	0.574	0.136	0.604	0.657
SizeofPCRproducts(bp)	300-3000	150-500	300-3000	150-3000	300-3000	150-3000	150-3000

Table 3: Nei'sgeneticdiversityandShannon'sinformationindexvalueswithinthespecies.

*Momordica*sp. Name	Sample size	Numberof polymorphic loci(%)	Nei'sgenetic diversity		Shannon's informationindex		HsandHt*	
			Mean	St.Dev	Mean	St.Dev	Mean	St.Dev
M.charantia	8	153 (25.37)	0.0999	0.1838	0.1454	0.2616	0.0999	0.0338
M.cochinchinensis	7	274 (45.44)	0.1572	0.1959	0.2357	0.2811	0.1572	0.0384
M.dioica	7	235 (38.97)	0.1448	0.1999	0.2136	0.2854	0.1448	0.0400
M.balsamina	2	108 (17.91)	0.0742	0.1590	0.1803	0.2321	0.0742	0.0253
*Momordica*sp.	24	589 (97.68)	0.3136	0.1274	0.4808	0.1622	0.3511	0.0186

Htvalueisforcompletesetof24accessionsdepictingthediversityinthe setofaccessionsincludedinthestudy.

Theaboveresultsclearly distinguish bothmonoecious speciesfromthedioeciousonesandthespecies withinboth groups.Uniquemarkersdistinguishingboth thegroupsandsomespecies-specificmarkerscouldalsobe identifiedthroughthestudy(supplementary Table S6).Seventeenmarkerscouldbeidentified differentiating monoecious and dioecious species,while85ampliconsspecifictooneofthefour*Momordica*specieswereidentified**.**Theseresults highlightthepotentialofmolecularmarkersinidentifying accessionsatspecieslevelandaccessingthegenetic diversityofaccessionsbothwithinandacrossspecies.

Discussion

Morphologicalevaluationbasedonquantitativetraits

Severalspeciesofgenus*Momordica*occurinIndia.Amongthem*M.charantia,M.dioica,M.cochinchinensis*and *M.balsamina*aredistributedacrossvariousagro-climaticzonesofIndia,Theaccessionsincludedinthis studywere collectedfromtheir respective zones, representingfourspecies:twoeachofmonoecious and dioeciousnature(Table1).

Clusteranalysisbasedonquantitativetraits

Thedendrogramobtainedfromclusteranalysisofthedistinctmatrixformedthemeanvalueoffifteenquantitative traitsclearlyexhibitsdistinction amongaccession offour*Momordica*speciesrepresentedinthestudy.Further,*M. dioica*formsdistinctclusters whilethe other three speciesgroupin separatecluster formingdifferentgroupsofeach specieswithinthecluster.This couldprobablebe becausethetraits evaluatedduringthestudydidnotinvolvethose featuresof floralbiology. This clusteringpatternof speciescould not establishanycorrelationbetweenthe clusteringpatternswiththegeographicaldistributionoftheaccessions.Deyetal.(2006)alsocouldnotestablishany suchcorrelationfor*M.charantia*accessions.However,non-congruence oftheresultswithpreviousstudy(Deyet al.,2006)couldbeduetoinclusionofmorenumberofspeciesinthestudyandrecordingdifferentquantitativetraits foranalysis. TheresultsareinpartialcongruencewithBharathietal.(2012)onrelatedness ofspeciesdemonstrated throughcrossability.

Themorphologicaltraitsrecordedinthisstudy,couldefficientlyclusterallthe accessionsrepresenting a species,thesetraitscanbehighlyeffectiveindiversityanalysis ofasetofgermplasmandindevelopingcoresetof thegermplasmrepresentingmaximumdiversityacrossthespecies.Kumaretal.(2010)haveadvocated useof strategyofidentifyinghighlyvariabledescriptorsforcapturingmaximumgeneticdiversityinthecoreset. Among*M.charantia*accessions,twogynoecious lines(Gy-323,Gy-333)recordedearlydaystoflower anthesisanddaystoediblematurityindicatingearlinessandearlyharvest.Theselinesalsoexhibitmaximumfruit perplant,fruitweightandyieldperplantindicatingtheirpotentialtodevelophighyieldinggynoeciousvarietyin futurebreedingprogramme (Rametal.,2002,Deyetal., 2006,Beheraetal., 2008 and Shukla et al., 2015).However, plants respondandadaptdifferentlytoselectionpressuresimposedbydistinctagroclimaticzones(Singhetal.,1998). Theseresultshighlightthepotentialofquantitativetraitsindistinguishingaccessionatspecieslevel.Rasuletal., 2007alsoreportedsimilardistinctionsofspeciesusingRAPDmarkers.

Molecularvariationandspeciesrelationships

Thegeneticrelationshipamongthefourspeciesis assessedbasedonJaccard'ssimilaritycoefficientfromthe pooleddatafrom differentmarkersystemsanddendrogramisgeneratedusingun-weighed pairgroupmethodwith arithmeticaverages(UPGMA). Therobustness ofnodesinthedendrogramisassessedbybootstrapanalysisby analyzingthedatawith1000replications(Fig.2)usingFreetreeandTreeviewsoftwares.Theperusalofthe dendrogramshowstwodistinctclustersofmonoeciousanddiociousspecieswithbootstrapvalueof100atnode. Thefirstmajorclusterincludestheaccessionfrom*M.cochinchinensis*with*M.dioica*specieswhilethesecond consistsofaccessionfrom*M.charantia*with*M.balsamina*. Theresultisalsosupportsthediversity analysisof plastids,mitochondriaandnuclearDNA,where*M.dioica*and*M.cochinchinensis*arenestedinaclade(Schaefer andRenner,2010).Thebootstrapvaluesatthenodesdifferentiating speciesarehightoalevelof100for*M. cochinchinenesis*and*M.dioica*,*M.charantia*and*M.balsamina*.Theclusteringpatternclearlydivides4groups, eachrepresenting asinglespecies includedinthisstudy.Thebootstrapvaluesrangesfrom 37to100for*M. cochinchinensis*;22to100for*Mdioica*and40to100for*Mcharantia*representinghighlevelofdiversityin accessionofthesespecies.The twoaccessionof*M.balsamina*includedin thisstudywerehighlydiversefromeach other withbootstrapvalueof100 for nodeconnectingthem.

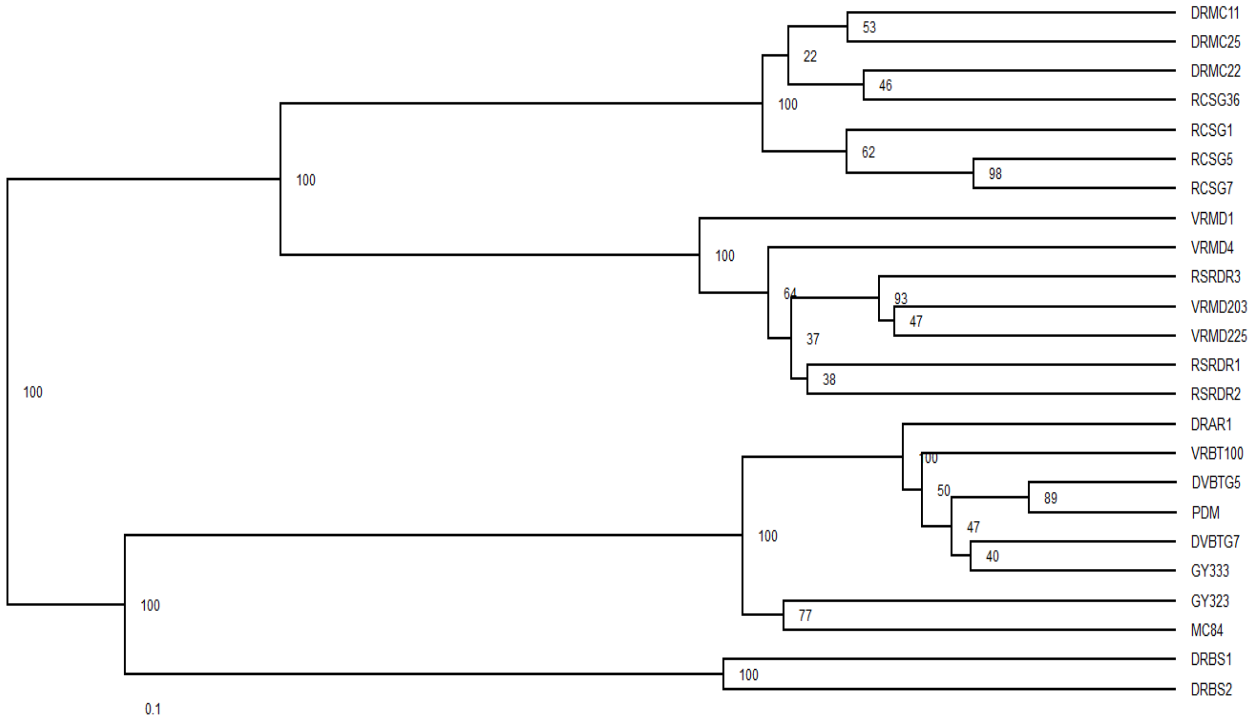

Figure2:UPGMAdendrogramof*Momordica*accessionswithbootstarpvaluesfromJaccard'ssimilarity coefficientobtainedfrommoleculardataanalysis.

Theperusalofdendrogramgeneratedfromthemoleculardatathrough1000replicationforbootstrap revealsresolution offourdistinctclusterseachrepresenting aspecieswithintermediate tohighbootstrapvaluesat thenodesrevealinghighgeneticheterogeneity amongtheaccessionsofthespecies.Similarresultsofhighgenetic diversitywithinspecieshavebeenreported(Deyetal.,2006;Rasuletal.,2007;Beheraetal.,2008;Dalamuetal., 2012andJietal.,2012).

Basedonmoleculardata,101primersgenerated600 scorableamplicons across fourspecies, ofwhich586polymorphic ampliconsexhibiting 97.08% polymorphismamongthesamples.This high level of polymorphismcan beattributedtotheaccessionsrepresenting diversespeciesinthestudy.Theresultsarenotincongruencewithearlierstudies(Deyetal.,2006;Beheraetal., 2008andDalamuetal.,2012)duetonon-inclusionofaccessionsrepresentingsuchdiversespecies.Rasuletal. (2007)includedoneaccessionof*M.dioica*asanout-groupto studydiversityin*M.charantia*usingRAPDmarkers.

Seventeen markerscouldbeidentifieddifferentiating monoecious and dioeciousspecies, while85 amplicons specifictooneofthefourMomordicaspecieswereidentified**.**Theseresultshighlightthepotential of molecularmarkersinidentifyingaccessions atspecieslevelandaccessing thegeneticdiversityofaccessions both withinandacrossspecies.Theperusalofdendrogramgeneratedfromthemoleculardatathrough1000replication forbootstraprevealsresolutionoffourdistinctclusterseachrepresenting aspecieswithintermediatetohigh bootstrapvaluesatthenodesrevealinghighgeneticheterogeneity amongtheaccessions ofthespecies.Similar resultsofhighgeneticdiversitywithin specieshave been reported(Deyetal.,2006; Rasuletal., 2007; Beheraetal., 2008andJietal.,2012).

Inthepresentstudy,morphological diversity basedonquantitativetraitsandmoleculardiversityshowedsome correspondenceintheclusteringpattern ofaccessionsrepresentingaspecies.Allthespeciescouldbedistinguished clearly, whilemoretraitsrelatedtofloralbiologyofthespecieswererequiredto distinguish species morphologicallyinthestudy.Thepotentialityofgynoeciouslinesforearlinessandyieldattributesisevidentand canbeeffectively exploitedinfuturebreedingprograms.Thoughaccessions ofsamespeciesgroupedtogetherin bothmorphological andmolecularanalysis,molecularmarkersweremoreefficientthanmorphologicalparameters innotonlydifferentiatingtheaccessionsat specieslevelbutalsodistinguishingmonoeciousanddioeciousspecies.

Supplementary Table

Supplementary Table S1: Morphologicalperformanceof*Momordica*sp. genotypesmeasuredfor15quantitativetraits

Accessions	Days to flower anthesis (Days)	Edible maturation	Branches /Plant	Plant height (cm)	Fruit diameter (cm)	Fruit length (cm)	Fruit /Plant	Fruit weight (g)	Seeds /Fruit	Petiole lenght(cm)	Pedicle length (cm)	Node to1st male flower	Node to1stf emale flower	Internodal length (cm)	Yield /Plant (kg)
Mcharantia															
GY-323	34.2	44.2	9	95.3	2.96	15.29	20.75	44.5	15	5.6	9.8	7.2	11.3	10.2	1.98
GY333	32.5	40.5	11	110.5	2.65	18.75	16.25	38.35	12	6.3	10.2	8.3	12.1	9.5	1.85
DRAR-1	47.3	52.6	7	115.4	3.33	16.66	14.47	36.56	16	5.4	9.5	9.4	13.2	11.2	1.26
VRBT-100	39.4	49.8	13	125.2	3.47	15.33	13.65	34.06	18	5.1	8.7	7.6	10.5	8.9	1.43
MC-84	41.7	52.2	10	113.4	3.12	10.25	15.23	35.55	14	3.2	9.1	8.8	12.5	7.9	1.66
DVBTG-7	45.5	50.5	9	145.2	3.26	12.6	11.82	40.25	16	4.6	12.2	5.6	9.8	9.9	1.82

DVBTG-5	42.3	54.1	15	153.6	2.62	18.98	12.65	30.28	12	5.5	10.5	7.8	10.2	11.3	1.34
PDM	49.1	58.6	6	114.5	4.11	9.55	10.52	35.32	13	6.2	13.9	9.4	13.7	10.5	0.98
M.cochinchinensis															
RCSG-1	58.5	73.6	20	190.5	4.35	7.52	24.55	112.55	20	4.9	15.6	18.3	25.4	15.5	2.25
RCSG-7	55.5	70.2	18	210.5	3.98	6.53	36.58	89.54	19	3.7	13.2	20.1	23.5	12.3	1.98
RCSG-5	63.2	81.5	19	165.3	4.85	5.14	21.35	96.25	18	5.6	14.6	21.4	27.4	15.7	1.45
RCSG-36	75.8	90.5	19	144.5	4.65	4.95	18.45	114.56	16	5.9	15.8	19.8	26.5	11.2	2.10
DRMC-22	68.9	88.6	14	135.6	3.25	5.11	16.87	126.35	24	4.5	15.6	14.5	18.4	14.7	1.56
DRMC-25	79.5	101.2	21	154.8	3.88	4.68	21.54	118.47	19	4.7	12.1	11.1	18.5	13.6	1.34
M.dioica															
VRMD-1	50.5	65.4	11	119.8	2.64	4.54	32.54	12.54	19	3.6	13.4	12.5	14.7	11.2	0.36
RSR/DR-1	54.6	68.5	13	165.6	2.95	4.62	39.21	19.58	16	3.3	8.9	10.2	13.5	8.6	0.45
RSR/DR-2	60.2	75.6	9	114.5	2.54	4.35	18.95	16.54	18	3.2	9.5	9.8	12.4	9.5	0.54
RSR/DR-3	41.2	62.1	14	110.5	2.22	4.29	26.56	14.25	24	2.1	9.7	8.7	10.4	7.2	0.74
VRMD-20-3	43.2	66.3	10	113.2	3.24	4.15	41.25	11.24	16	3.9	6.4	12.5	16.5	6.7	0.65
VRMD-22-5	55.8	62.5	16	145.6	3.11	5.11	35.89	10.87	13	4.5	7.8	9.6	13.2	8.5	0.55
DRMC11	60.5	86.2	19	169.5	3.26	8.53	19.55	115.64	14	3.7	8.4	8.5	12.4	4.9	1.56
VRMD-4	55.4	68.5	12	129.6	2.98	3.65	40.25	18.11	11	4.9	7.8	9.1	11.2	5.5	0.48
M.balsamina															
DRBS-1	35	44.3	6	65.8	1.98	5.82	10.25	28.51	9	3.6	6.8	6.5	8.5	5.8	0.58
DRBS-2	41	53.6	9	85.3	1.85	6.89	13.65	30.55	12	2.4	9.4	5.4	6.7	3.5	0.85
Mean	51.31	65.05	12.92	133.07	3.22	8.47	22.20	51.27	16.00	4.43	10.78	10.92	14.68	9.74	1.25
S.D.	12.62	16.16	4.60	33.19	0.78	5.02	10.08	40.48	3.80	1.16	2.86	4.62	5.74	3.25	0.60

Supplementary Table S2:DetailsofRAPDprimersselectedforthe study

Primers	**Sequence5`to3`**	**AT(**℃**)**	**GC (%)**	**Total bands**	**Polymorphic bands(%)**	**Rp**	**PIC**
OPAF-04	TTGCGGCTGA	33	60	8	8 (100)	5.667	0.749
OPAF-06	CCGCAGTCTG	33	70	4	3 (75)	2.167	0.651
OPAF-11	ACTGGGCCTC	34	70	4	4 (100)	2.917	0.613
OPAF-12	GACGCAGCTT	33	60	4	4 (100)	2.250	0.723
OPAF-15	CACGAACCTC	33	60	3	3 (100)	0.167	0.786
OPAG-03	TGCGGGAGTG	33	70	3	3 (100)	1.833	0.865
OPAG-04	GGAGCGTACT	33	60	3	3 (100)	2.167	0.860
OPAG-06	GGTGGCCAAG	33	70	4	4 (100)	2.833	0.849
OPAG-10	GGTTGGAGAC	33	60	7	7 (100)	3.750	0.686
OPAG-11	TTACGGTGGG	34	60	4	4 (100)	2.417	0.749
OPAG-12	CTCCCAGGGT	34	70	4	4 (100)	3.667	0.706
OPAG-15	CCCACACGCA	34	70	4	4 (100)	2.500	0.715
OPC-06	GAACGGACTC	33	60	3	3 (100)	3.000	0.856
OPC-08	TGGACCGGTG	34	70	5	5 (100)	1.917	0.619
OPC-14	TGCGTGCTTG	33	60	3	3 (100)	5.167	0.760
OPC-16	CACACTCCAG	33	60	8	8 (100)	2.333	0.940
OPF-16	GGAGTACTGG	33	60	5	5 (100)	4.583	0.542
OPH-10	CCTACGTCAG	34	60	7	7 (100)	2.500	0.396
OPK-20	GTGTCGCGAG	33	70	5	5 (100)	4.167	0.843
OPL-07	AGGCGGGAAC	33	70	6	6 (100)	1.167	0.985
OPL-08	AGCAGGTGGA	33	60	5	4 (100)	4.306	0.839
OPL-14	GTGACAGGCT	33	60	6	6 (100)	3.500	0.793
OPL-16	AGGTTGCAGG	33	60	7	7 (100)	4.917	0.654
OPL-20	TGGTGGACCA	33	60	10	9 (90)	5.833	0.795
OPM-12	GGGACGTTGG	33	70	7	7 (100)	4.167	0.788
OPM-14	AGGGTCGTTC	34	60	6	6 (100)	1.083	0.237
OPN-08	ACCTCAGCTC	33	60	4	4 (100)	1.500	0.832
OPN-12	CACAGACACC	33	60	4	4 (100)	3.083	0.724
OPN-14	TCGTGCGGGT	33	70	4	4 (100)	3.250	0.870
OPO-06	CCACGGGAAG	33	70	6	6 (100)	4.167	0.792
OPO-10	TGTGCCCGAA	33	60	6	6 (100)	1.750	0.947
OPY-03	ACAGCCTGCT	33	60	4	4 (100)	5.500	0.758
OPY-04	GGCTGCAATG	33	60	7	7 (100)	2.917	0.740
OPY-06	AAGGCTCACC	34	60	4	4 (100)	5.417	0.733
OPY-07	AGAGCCGTCA	33	60	7	7 (100)	5.250	0.770
OPY-08	AGGCAGAGCA	33	60	8	8 (100)	2.583	0.928
OPY-09	AGCAGCGCAC	34	70	5	5 (100)	2.833	0.701
OPY-14	GGTCGATGTG	33	60	4	4 (100)	2.333	0.592
OPY-15	AGTCGCCCTT	34	60	4	4 (100)	2.917	0.639
OPY-16	GGGCCAATGT	34	60	5	5 (100)	1.917	0.634
OPY-17	GACGTGGTGA	33	60	3	3 (100)	5.500	0.717
OPY-18	GTGGAGTCAG	33	60	8	8 (100)	2.750	0.857
OPZ-01	TCTGTGCCAC	33	60	5	5 (100)	4.250	0.767
OPZ-06	GTGCCGTTCA	33	60	4	1 (25)	2.583	0.590
OPZ-11	GGGTCTCGGT	33	70	6	6 (100)	3.417	0.724
OPZ-12	TCAACGGGAC	34	60	2	2 (100)	1.333	0.889
OPL-19	GAGTGGTGAC	33	60	5	5 (100)	3.917	0.841
OPZ-13	GACTAAGCCC	33	60	8	8 (100)	4.167	0.911
OPZ-14	TCGGAGGTTC	34	60	5	2 (100)	2.500	0.907
OPL-18	ACCACCCACC	33	70	11	11 (100)	5.417	0.895
				264	255 (96.59)		

Supplementary Table S3:DetailsofISSRprimersselectedforthe study

Primers	Sequence5'to3'	Tm (°C)	GC (%)	Total bands	Polymorphic band(%)	Rp	PIC
UBC880	GGAGAGGAGAGGAGA	55.6	60	14	14(100)	6.083	0.862
UBC834	AGAGAGAGAGAGAGACYT	54.5	47	10	10(100)	5.333	0.873
UBC856	ACACACACACACACACCTA	55.6	47	12	11(91.66)	5.833	0.651
UBC810	GAGAGAGAGAGAGAGAT	54.5	47	10	10(100)	4.167	0.727
UBC840	GAGAGAGAGAGAGAGAYT	57.5	47	9	9 (100)	5.333	0.881
UBC843	CTCTCTCTCTCTCTCTRA	53.2	47	9	9 (100)	4.083	0.906
UBC811	GAGAGAGAGAGAGAGAC	60.0	53	8	8 (100)	3.250	0.892
UBC888	BDBCACACACACACACA	60.0	50	12	12(100)	4.917	0.802
UBC861	ACCACCACCACCACCACC	60.0	67	10	10(100)	7.000	0.860
UBC835	AGAGAGAGAGAGAGAGYC	54.5	53	9	8 (88.88)	3.833	0.820
UBC825	ACACACACACACACACT	50.0	47	11	11(100)	3.667	0.825
UBC866	CTCCTCCTCCTCCTCCTC	54.5	67	10	10(100)	5.667	0.732
UBC841	GAGAGAGAGAGAGAGACTC	57.5	53	9	9 (100)	4.750	0.802
UBC813	CTCTCTCTCTCTCTCTT	54.5	47	8	8 (100)	5.833	0.883
UBC887	DVDTCTCTCTCTCTC	60.0	50	7	6 (85.71)	4.833	0.794
UBC822	TCTCTCTCTCTCTCTCA	54.5	47	9	9 (100)	4.917	0.824
UBC892	TAGATCTGATATCTGAATTCCC	53.2	36	9	9 (100)	1.667	0.534
UBC855	ACACACACACACACACCTT	57.5	47	9	9 (100)	6.417	0.865
UBC884	HBHAGAGAGAGAGAGAG	53.2	50	5	5 (100)	2.833	0.828
UBC848	CACACACACACACACAAGG	57.5	53	7	7 (100)	9.500	0.731
UBC842	GAGAGAGAGAGAGAGAYG	60.0	53	8	8 (100)	7.083	0.783
UBC890	VHVGTGTGTGTGTGTGT	57.5	50	8	8 (100)	4.083	0.844
UBC824	TCTCTCTCTCTCTCTCG	54.5	53	6	6 (100)	5.083	0.787
UBC853	TCTCTCTCTCTCTCTCRT	55.6	47	7	7 (100)	6.250	0.781
BC854	TCTCTCTCTCTCTCTCAGG	57.5	53	6	6 (100)	3.500	0.869
UBC891	HVHTGTGTGTGTGTGTG	57.5	50	5	5 (100)	5.333	0.768
UBC809	AGAGAGAGAGAGAGAGG	57.5	53	5	5 (100)	5.250	0.712
UBC815	CTCTCTCTCTCTCTCTG	54.5	53	7	7 (100)	6.583	0.830
UBC836	AGAGAGAGAGAGAGACYA	54.5	47	7	7 (100)	5.500	0.867
BC850	GTGTGTGTGTGTGTGTCTC	60.0	53	5	5 (100)	4.500	0.720
UBC812	GAGAGAGAGAGAGAGAA	50.0	47	3	3 (100)	2.583	0.607
UBC814	CTCTCTCTCTCTCTCTA	54.5	47	6	6 (100)	5.417	0.562
UBC886	VDVCTCTCTCTCTCTCT	54.5	50	5	5 (100)	5.750	0.800
UBC844	CTCTCTCTCTCTCTCTRG	50.0	53	4	4 (100)	2.417	0.898
UBC895	AGAGTTGGTAGCTCTTGATC	57.5	45	4	4 (100)	4.250	0.898

R-Wobble(A+G),H-Wobble(A+C+T),Y-Wobble(C+T),Y~Q-3'Wobble(C+T),D-Wobble (A+G+T),V-Wobble(A+C+G),B-Wobble(C+G+T)

Supplementary Table S4: DetailsofSSRprimers(Wangetal.,2010)selectedforthe study

Primers	PrimerSequence(5'…….3')	Tm (°C)	GC (%)	Total bands	Polymorphic bands(%)	Rp	PIC
S24	F:GCTCTGCGTTTCATTCTTCA R:TGAACCCTCAGACTCAAACTC	60	46	5	4 (80)	3.250	0.961
N6	F:GGGAATTCTCAAAGAGCCAGA R:TGGCACACTCTGCATGAAAT	57	46	4	4 (100)	0.750	0.786
N24	F:GCTCTGCGTTTCATTCTTCA R:TGAACCCTCAGACTCAAACTC	57	46	6	6 (100)	1.333	0.581
S13	F:TTGGTTGTGGTGCTGAGTTC R:GATGTAGGGGTTGGGTTGAT	57	50	5	5 (100)	3.917	0.864
	F:GTCTTCCAGGTTGGGAACAG	59	53	10	10(100)	0.653	0.859

N1	R: ATCTGGTTCCTCGGGAGATT						
S15	F:GGGTAGTGGAATGATGGGTT R:TAGTGTTTTCGTGAGGGAGG	57	50	5	5 (100)	1.917	0.886
S33	F: ATTTAGTGGGGCGGGTAGT R:TGGATGAGCATGTTAGGGATC	54	50	4	4 (100)	2.500	0.701
N9	F: ATCCATCCCCACAAGTTGAA R:CCATAAGGATATGTTTGCATGG	60	43	2	1 (50)	0.833	0.413
S18	F:GGGTAGTGGAATGATGGGTT R:TAGTGTTTTCGTGAGGGAGG	58	50	3	3 (100)	1.667	0.825
S32	F:CTAAATCACGCAAACCCATC R:GAGCAAAAGACTGAGGAAAACT	54	43	4	4 (100)	3.250	0.891
S9	F:TTCCCATTCACAGATCACTCC R:CCACCAAATTCAAGAACCCAC	57	48	2	2 (100)	1.500	0.734
N5	F:CGTCGCTCTCACAAGAGATAAG R:TTTGGTGGAAATCCCCTATT	59	45	2	2 (100)	1.333	0.722
S20	F:CCCCTTCTAATCACAACCAA R:GGCCTAATTTCTGCCCTTT	58	46	3	3 (100)	1.917	0.886
N12	F:CAGAGGGGTGGTTCCTCTTT R:CCACATGGATGATCGAGAGA	59	53	3	3 (100)	2.250	0.920
S12	F:CAGAGGGGTGGTTCCTCTTT R:CCACATGGATGATCGAGAGA	57	53	3	3 (100)	2.083	0.814
S26	F:GAACGCCCTGTGACTTTAGC R:TTTCGTCTTCCAATGAGCC	58	51	2	2 (100)	2.750	0.829
				63	**61(95.62)**	**1.993**	**0.792**

Supplementary TableS5: Nei'scoefficientfor geneticdiversity(abovediagnol)andNeiand Licoefficientfor geneticdistance (belowdiagnol)amongthespecies.

	M.charantia	*M.cochinchinensis*	*M.dioica*	*M.balsamina*
M.charantia	---	0.543	0.5728	0.7085
M.cochinchinensis	0.6107	---	0.7362	0.6924
M.dioica	0.5572	0.3111	---	0.7062
M.balsamina	0.3446	0.3675	0.3479	---

Supplementary Table S6: Primerswithspecies-specificbandswiththeirmolecularsize

Uniquemarkers	***Momordica*speciesidentified with molecularsize(bp)**
RAPDPrimers	
OPM-14	*M.dioica*(650), *M.cochinchinensis*(900)
OPL-07	*M.dioica*(950)
OPL-08	*M.cochinchinensis*(850)
OPAF-12	*M.charantia*(400)
OPH-10	*M.dioica*(750), *M.charantia*(400)
OPAG-012	*M.charantia*(500), *M.cochinchinensis*(950)
OPAF-04	*M.cochinchinensis*(890)
OPAF-11	*M.charantia*(1250), *M.dioica*(350)
OPC-16	*M.charantia*(900)
OPC-08	*M.charantia*(850)
OPZ-11	*M.balsamina*(1300)
SSRsPrimers	
N01	*M.charantia*(200,250)
N24	*M.charantia*(320)
S13	*M.charantia*and*M.cochinchinensis*(220)
S32	*M.charantia*(500)
S18	*M.dioica*(240)

S33	*M.dioica*(425)
S33	*M.charantia*and*M.balsamina*(380)
S33	*M.cochinchinensis*and*M.dioica*(250)
ISSRMarkers	
UBC866	*M.charantia*(700,1300), *M.dioica*(800), *M.charantia*and*M.cochinchinensis*(550)
UBC822	*M.charantia*(700,900,1400), *M.dioica*(1100), *M.cochinchinensis*(330,400,800), *M.charantia*and*M.dioica*(550)
UBC834	*M.dioica*(1500), *M.charantia*(260,275,500), *M.cochinchinensis*(250)
UBC887	*M.charantia*(1500), *M.dioica*(700,750,1200), *M.cochinchinensis*(700), *M.dioica*and*M.balsamina*(500)
UBC842	*M.charantia*(500,680,1200,1500), *M.dioica*(500,750), *M.cochinchinensis*(700), *M.charantia*and*M.balsamina*(480)
UBC836	*M.dioica*(1000), *M.cochinchinensis*(540,800), *M.dioica*and*M.balsamina*(275,500), *M.balsamina*(260)
UBC886	*M.charantia*and*M.balsamina*(1000), *M.dioica*(750,900), *M.cochinchinensis*(600), *M.charantia*(500)
UBC888	*M.charantia*(2400), *M.cochinchinensis*(250,2000), *M.dioica*(250,1000,1700)
UBC890	*M.cochinchinensis*(500,1500), *M.dioica*(660), *M.balsamina*(430)
UBC840	*M.charantia*and*M.cochinchinensis*(250)
UBC855	*M.charantia*(2500), *M.cochinchinensis*(575)
UBC841	*M.cochinchinensis*(280), *M.dioica*(250)
UBC848	*M.charantia*and *M.dioica*(1500), *M.cochinchinensis*and*M.dioica*(900), *M.dioica*(500)
UBC812	*M.cochinchinensis*and *M.balsamina*(300)
UBC825	*M.balsamina*(590,1270), *M.charantia*(530,1100), *M.cochinchinensis*and*M.dioica*(645), *M.dioica*(600)
UBC861	*M.dioica*(1400), *M.cochinchinensis*(300,400,750,900), *M.dioica*(300,335)
UBC892	*M.cochinchinensis*and*M.dioica*(300,1600)
UBC843	*M.dioica*(800), *M.cochinchinensis*and*M.dioica*(400)
UBC884	*M.charantia*(200,600), *M.charantia*and*M.dioica*(530)
UBC855	*M.cochinchinensis*and*M.dioica*(200,700,750), *M.charantia*(450), *M.cochinchinensis*(300)
UBC891	*M.charantia*(250)
UBC815	*M.dioica*(800,1300), *M.charantia*(900,1200)
UBC809	*M.cochinchinensis*(600), *M.charantia,M.dioica*and *M.balsamina*(425,500), *M.dioica*(300)
UBC891	*M.cochinchinensis*and*M.dioica*(750), *M.cochinchinensis*(300)
UBC844	*M.charantia*(900), *M.cochinchinensis*and*M.dioica*(500)
UBC880	*M.dioica*(1300), *M.charantia*(335,750), *M.cochinchinensis*(573)
UBC856	*M.cochinchinensis*(300,1300), *M.dioica*(250), *M.charantia*and*M.balsamina*(240)
UBC853	*M.charantia*and*M.balsamina*(1500), *M.dioica*(750,1100), *M.cochinchinensis*(500)
UBC813	*M.dioica*(1300), *M.cochinchinensis*(1000), *M.charantia*(400)
UBC810	*M.cochinchinensis*(1200), *M.charantia*(250,275,730), *M.balsamina*(250)
UBC814	*M.cochinchinensis*and*M.dioica*(400)
UBC835	*M.charantia*and*M.balsamina*(200,1000), *M.charantia*(600), *M.dioica*(250)
UBC811	*M.charantia*(1600), *M.cochinchinensis*and*M.dioica*(1500), *M.charantia*and *M.dioica*(300,635), *M.dioica*(700)

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Conflict of Interest

The authors declared no conflict of interest.

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