Possibility for Exploitation and Identification of Rice Yield and Seed Quality Orthologs in Peanut (Arachis hypogaea L.)

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Abstract

Background

The progress in tagging/mapping of genes in crops like *Arabidopsis*, rice, maize etc. is far ahead when compared to the major legume crops *viz*. groundnut, chickpea, redgram, greengram, soyabean etc., even in the post release period of legume genome sequence databases. This can be attributed mainly to low level of available molecular genetic diversity in these crops.

Methods

The study of transferability of Rice yield gene tagged markers to Peanut was carried out by employing17 diverse groundnut genotypes. ANOVA revealed significant differences for six yield and seven seed quality traits studied which indicates availability of ample amount of variability among the genotypes. Molecular analysis was conducted to analyse the transferability of known rice yield, grain size and micronutrient content (Fe and Zn) controlling gene tagged markers (GTMs) to peanut by using 45 GTMsthat targets 24 known functional genes.

Results

Of 45 markers, 31 (76%) were transferable to peanut, denotes very high conservation at functional regions. The extent of amplification of rice GTMs at individual groundnut genotype level was observed from 79.17% for the cultivated varieties (2n = 4x) Nithya Haritha, Greeshma, Prasuna, Kalahasti, Narayani and with a wild genotype, *Arachisvillosa*(2n = 2x) to 91.67% for Dheeraj variety. The analysis on transferable efficiency of individual rice GTM revealed that 17 markers belong to 14 rice genes showed amplification among all the 17groundnut genotypes (100%) under study.

Conclusions

Hence, focusing research on the available knowledge of functionally characterized genes of molecular model crops and vast list of annotated orthologous genes present in 'Omics' databases, widens the scope to tag the genes at molecular level and thereby to improve the cropslike groundnutthat has meager progress in gene tagging;by pyramiding of desirable genes with high veracity.

Introduction

Peanut (*Arachis hypogaea* L.) belongs to *Papilionaceae* subfamily of the *Fabaceae* family which is an allotetraploid (AABB; 2n = 4x = 40). It is most preferable as an excellent source ofnutrition to both human and also animals due to its high content of digestible proteins (22–30%), vitamins (E, K and B group), minerals (phosphorus, calcium, magnesium and potassium) and phytosterols.

The yield level of many crop plants including groundnut is stagnated during post 'Green Revolution' era which threatens about the food security in near future wherein it needs to achieve 68 million tonsof groundnut projection by 2050 (Alexandratos and Bruinsma, 2012) with a productivity growth rate of about 4–5% (DGR Vision 2050). To break this stagnation and to attain the future projections there is a massive need to achieve rapid success in crop improvement programmes. At this juncture, apart from conventional crop improvement methods what would benefit a breeder during post genome sequencing era is, identification of trait governing genes and there by tagging of functional variants at molecular level.

The progress in tagging/mapping of genes in crops like *Arabidopsis*, rice, maize etc. is far ahead when compared to the major legume crops *viz*. groundnut, chickpea, redgram, greengram, soyabean etc., even in the post release period of legume genome sequence databases. This can be attributed mainly to low level of available molecular genetic diversity in these crops.

In groundnut although there is availability of high level of morphological diversity among varieties of *A. hypogaea*, this has not been generally reflected in the level of detectable molecular genetic diversity. Thus, slow progress of genomics in peanut is attributed especially to its tetraploid nature besides low marker polymorphism (Burrow et al., 2001). Peanut being allotetraploid, consists of A and B sub-genomes that are originated in its diploid progenitors *Arachis duranensis*(A-genome) and *Arachis ipaensis*(B-genome), respectively. The genome sizes of the two wild species are 1.25 Gb and 1.56 Gb, respectively. A high-quality peanut (*A. hypogaea*) genome sequence contains 2.54 Gb with 20 pseudomolecules and 83,709 protein-coding gene models (Zhuang et al., 2019). Mapping of genes *de novo* either by forward genetic approaches or by reverse genetic approaches takes lot of time and labor which further makes difficult with ploidy level.

Comparative mapping reveals high degree of sequence co-linearity across the plant kingdom among related species and as well genera, thus helps to exchange molecular resources between them. Further, the sequenced databases of several crop plants indicate homology existing between the highly conserved regions during their course of evolution, especially the genic regions that encompass motif/domains of particular gene family. These genes are said to be orthologous/paralogous to each other and known to participate in the same function(s) in plant system. For instance, a homolog gene was identified in tomato (ovate gene) which has a similar function of putative transmembrane protein in rice for grain size (*GS3*) (Gupta et al., 2006). A homolog in maize for thick tassel dwarf (*td1*) has been identified having a similar function of LRR (leucine rich repeat) receptor like protein in *Arabidopsis* (*clv1*) (Gupta et al., 2006).

Hence, the orthologous genes of model crops viz. *Arabidopsis*, rice can become a rich and flexible source, which allow further identification of their othologous genes in under researched legume crops like greengram, blackgram, groundnut etc., there by usage of these gene's functional variants to food security in near future of climate resilient era through precise crop improvement breeding. Thus, there is large possibility for transferability of known gene targeted markers from one crop genome to other. For instance, from Triticale, 58% and 39% transferability of molecular markers was achieved for wheat and rye, respectively (Kuleung*et al*., 2004). Further, it is proved that the seed size or weight governing genes are extremely orthologs among rice, maize, and *Arabidopsis* (Li et al., 2010). Interestingly, the transferability of genic microsatellite (EST-SSR) markers has been proved from grass family member-*Sorghum bicolor* to the legume member-Groundnut (Siddannaet al., 2012). These findings give strength to the concept of benefit in improving under-researched legume crops with the information obtained from model crop genomes. Hence, the knowledge of known yield and stress governing genes can be deployed to tag the orthologs in these orphan legume crops wherein it facilitates Marker Assisted Breeding with high veracity for the targeted traits.

Materials And Methods

Plant material consists of a total of 17 groundnut genotypes released from RARS, Tirupati, Acharya N.G. Ranga Agricultural University (ANGRAU)and TAG-24, a popular variety released from BARC,Mumbai(Fig. 1 and SupplementaryTable 1). A popular rice variety NLR34449 (Nellore Mahsuri) developed from cross IR72 × BPT 5204, is a fine grain short duration (120–125 days) variety possessingBlast resistance that released from ARS, Nellore, ANGRAU in the year 2009 was used as control variety for molecular studies. Two wild relatives of groundnut i.e.*Arachis glabrata* and *Arachis villosa*that are being maintained at RARS, Tirupati were used in the study as diploid wild source with main emphasis to observe the molecular level similarities/changes with respect to alloploid groundnut at the targeted loci.

Phenotyping

Observations on seven quantitative and six qualitative parameters *i.e.*, Plant height (cm), number of primary branches per plant, number of secondary branches per plant, pod yield per plant (g),100 pod weight (g), 100 kernel weight (g), shelling percent, oil content (%) (Infratec™ 1241 Grain Analyzer, Denmark), protein content (%) (Infratec™ 1241 Grain Analyzer, Denmark), total sucrose content (µg g^{-1}) (Sadasivam and Manickam, 1996), total free amino acids (µg g^{-1}) (Sadasivam and Manickam, 1961), total soluble sugars (g g^{-1}) (Sadasivam and Manickam,1996)and seed Fe and Zn content (ppm) (using Atomic Absorption Spectrophotometer, AAS)were recorded for all the genotypes of groundnut separately on randomly chosen five competitive plants in each genotype, in each replication.

Molecular Study

Choice of markers:

A total of 45 markers targeting 24 number of rice yield, grain size and micronutrient content (Fe and Zn) controlling genes were selected that were used in the rice crop improvement through marker assisted breeding programmes (SupplementaryTable 2).

Micronutrient content (Fe and Zn) controlling genes i.e., YSL gene specific *Indels* were identified from RiceVarMap database (http://ricevarmap.ncpgr.cn/) and markers were designed by providing the specific ID of each*Indel* (e.g. vf0226164188, vf0226169288, vf0226164382) in the suit (http://ricevarmap.ncpgr.cn/primer_design_id/) wherein it consists of Primer3 as a backend engine.To design SSR markers, gene sequences for LOC02g43410 (YSL15),LOC04g44300(YSL13) with an additional 1kb sequence both upstream and downstream were downloaded from rice genomic database of Gramene (http://www.gramene.org). The microsatellite region of candidate genes were identified using SSRIT tool (http://archive.gramene.org/db/markers/ssrtool) and then primer designing was done using primer3 v.4.0.0, a primer designing tool (http://bioinfo.ut.ee/primer3-0.4.0/).

DNA Preparation And PCR Protocol

Total DNA from 17 groundnut cultivated varieties, two wild genotypes viz. *Arachis glabrata* and *Arachisvillosa*and a rice genotype NLR34449 (Nellore Mashuri) was isolated using CTAB protocol of Lin *et al*.(2001) and purified using 3M sodium acetate. The DNA of both groundnut and rice genotypes were assessed for their purity and intactness using the 0.8% agarose gel and as well the NanoDrop spectrophotometer.

Initially rice DNA was subjected to Polymerase Chain Reaction (PCR) by using chosen list of rice yield and grain quality controlling gene targeted markers. PCR reaction was performed in a gradient thermal cycler (Eppendorf, Germany) in a 10.0µl volume of mix containing 1.0 µl of 10X *Taq* buffer with $MgCl_2$, 1.0 µl of 10mM dNTP mix, 0.1 µl of *Taq* DNA polymerase (5U/µl), 0.5 µl each of 10pm forward and reverse primers, 2.0 µl of 100ng/µl genomic DNA and 4.9 µl of autoclaved millipore water.PCR optimisation was carried out with initial denaturation of 94°C, followed by the reaction mix was subjected to 35 PCR cycles, wherein each cycle consisting a denaturation of 94°Cfor 30 sec, annealing of 50–65˚C for 30 sec, extension of 72˚C for 30 sec and final extension of 72˚C for 10 min. Annealing temperature of each primer was standardized by doing PCR with the temperature range of 50–65˚C, to assure strong and clear amplification. Later the primers were employed for amplification of DNA that isolated from groundnut genotypes. The PCR products were resolved in 3% agarose gels and images were captured with Geldoc Imaging system.

Analysis Of Marker Transferability Level

The amplified alleles were scored for groundnut genotypes, with respect to the allelic sizes of rice using a standard marker i.e.50bp ladder. Percent Transferability of gene specific primers that can be from rice to groundnut genome was estimated trait/gene wise, based on the unambiguous amplification of markers at respective allele sizes. Further allele coding was assigned based on the presence of allele similar to rice.

Insilico Analysis Of Groundnut Genome For Rice Gene Orthologs

PeanutBase (https://peanutbase.org/home) was analysed for retrieving of rice yield and grain size/quality governing gene orthologs, to understand the extant of these genes' appearance and distribution at structural genome level. Primer BLAST was performed against peanut expressed sequence data of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi), to identify/support the functionality of these genes if any, in the genome.

Results

Analysis of Variance

Analysis of variance revealed significant differences among genotypes for all the characters studied at both 5% and 1% level except for zinc content which is significant at 5% level only (Table 1). Similar reports of significance for ANOVA were given for yield and quality traits in groundnut by Shankar et al. (2019) and Chandrasekhara *et al.* (2020). Presence of high phenotypic variability is important for the traits under a study, to identify genomic/functional variations if any at the targeted traits and thereby to tag a trait at molecular level.

Table 1
Analysis of variance for yield and seed quality traits in groundnut

S. No	Character	Mean Sum of Squares		
		Replications (df: 2)	Treatments (df : 23)	Error (df : 46)
1	Plant Height (cm)	133.08	298.827**	27.71
2	Primary Branches per Plant (No.)	2.94	4.243**	0.56
3	Secondary Branches per Plant (No.)	0.13	2.660**	0.08
4	Pod Yield per Plant (g)	4.97	30.948**	5.04
5	Hundred Pod Weight (g)	285.89	1329.038**	217.79
6	Hundred Kernel Weight (g)	36.15	144.416**	15.12
7	Shelling Percentage (%)	12.26	83.413**	16.33
8	Oil Content (%)	0.49	1.708**	0.06
9	Protein Content (%)	0.06	0.282**	0.07
10	Total Free Aminoacids (TFA)(µg/g)	6.31	22218.767**	64.76
11	Total Soluble Sugars (TSS)(g/g)	0.02	0.062**	0.00
12	Total Sucrose Content(µg/g)	1.38	264.623**	2.91
13	Fe Content (ppm)	134.11	4359.679**	357.81
14	Zn Content (ppm)	48.76	194.429*	91.88
*,** significant at 5% and 1% level respectively				

Standardization Of Markers With Rice

All the 45 markers that are targeted to 24 rice yield and quality trait governing genes selected for genotyping analysis were standardized primarily with rice genomic DNA of NLR34449 at various temperatures (temperature gradient) *i.e.* 50–65°C (**Supplementary Fig. 1**). Of 45 markers, 41 primers representing all 24 genes were amplified with NLR 34449(Table 2), four primers namely MOC1, ex Gn1a, exDep1 and C62 were not amplified with NLR 34449. The primers that amplified with rice were further tested for their transferability with groundnut genotypes.

Table 2
Exploitation of 45 rice gene tagged markers in the study and their allele sizes observed in groundnut with respect to rice

Trait reported in rice/considered to be studied in groundnut	Gene Name	Chr	Marker	Annealing temperature	SIZE OF ALLELE (bp)			
					Reported size of allele in Rice	Observed size (bp)		
						Rice	wild groundnut	cultivated groundnut
Plant height	sd1	1	sd1-h	55	843 (semidwarf-Habataki); 800 (normal plant introduced with sd1-Sashaniki)	843	190	NA
Plant Architecture	MOC1	6	MOC1	65**	1900(primer blast reference)	NA	NA	NA
	OsSPL14	8	OsSPL14	50	500 (Heavy panicle)	500	500	500
	Plant Architecture and Yield 1 (PAY1)	8	PAY1sp6	53	200 (Nipponbare)-primer blast reference	200	200	200
Yield Contributing Genes	Gn1a	1	ex Gn1a	50–65**	532(Habataki, ST12)-donor for MAB &highgrain number per panicle, 321bp(Parao)	NA	NA	NA
	Grain Number2 (GN2)	2	RM3535	58	185	185	185	185
	YLD	8	RM223	58	139–163 (identify aromatic and non-aromatic germplasm)	165	165	165
			HY2-4	50–65		900	NA	NA
	NAL1/SPIKELET NUMBER (SPIKE)	8	SPIKE-INDEL 3	62	151(CT805,Parao)-yield positive, NAL1- japonica allele,171	171	171	171
	EP3	2	S5 803	59	243 (erect panicle)	243	NA	NA
	Hybrid sterility LOC_Os06g11010	6	S5-1	59	321(02428) -wide compatibility var (heterosis and ideal plant type breeding),457(Nipponbare) - incompatible var	457	NA	NA
	SCM2/Aberrant Panicle Organization 1 (*APO1*)	6	SCM2-INDEL1	57	117 (Habataki)- high yield and lodging resistance, 105 (Parao)	117	117	117
	DEP1 (DENSE AND ERECT PANICLE),	9	exDep1	50–65**		NA	NA	NA
			DEP1 INDEL1	58	1031 (increased grain number per panicle), 406(Osmanick-Turkish high yielding variety)	1031	NA	NA
			DEP1-1	58	1235(Wuyunjing8) (erect panicle) –heterosis and ideal plant type breeding,1860(Nipponbare)-non-erect panicle	1860	NA	NA
			Dep1s7	59	127(primer blast reference)	127	127	127
Seed Quality Genes	GS2	2	RM3212	58	181(Nipponbare)- medium grain phenotype	181	181	181
	GS3	3	RGS1	55	180 (Improve Grain Size),200	180	180	180
			SR17	58	1000 (Improve Grain Size),1400	1400	NA	NA
	GS5	5	C62	50–65**		NA	NA	NA
			RM593	58	279(primer blast reference)	279	NA	NA
			GS5 INDEL1	55	67(ST6, Parao)- wide grain, 63 (NSIC Rc158)- medium grain	63	63	63

Trait reported in rice/considered to be studied in groundnut	Gene Name	Chr	Marker	Annealing temperature	SIZE OF ALLELE (bp)			
					Reported size of allele in Rice	Observed size (bp)		
						Rice	wild groundnut	cultivated groundnut
			RM574	58	240(Zhenshan97) (Oryza sativa var.indica) - grain width	240	240	240
	OsSNB, SUI4/SNB	7	OsSNB2	58	997(primer blast reference)	50	50	50
	GW2 (Grain weight)	2	GW2SNP2	58	51(Kasalath), TD70-31&21bp fragments- more valuable for grain width and weight	51	51	51
			GW004	57	750,1050- not much used for detecting allelic variations in GW2	750	NA	NA
	GW5 /SW5	5	RM3328	57	119(increase grain width)	119	NA	95
			RMw513	59	171(Nipponbare)- slender grain and low chalkiness	600	700	600
			N1212	59	759 (increased grain width) - Nipponbare, TD70	65	65	65
	GW7	7	RM22015	58	176(1000 grain weight)	176	176	176
	GLW7/OsSPL13	7	GLW7/OsSPL13/SPL13	62	140(primer blast reference)	140	140	140
			RM505	55	220(Sonasal) -short grain, 170(Chiguhong)-grain plumpiness,180(PB1121) - extra-long sender grain	500, 180	NA	400
			RM21945	55	292	292	292	292
	GW8	8	GW8 PRO2	58	861(primer blast reference)	861	NA	861
Shelling Percentage	GIF1	4	exGIF1	58	96(primer blast reference)	96	96	96
			RM16942	58	181	181	NA	135
Flowering Time	Hd1	6	Hd1	50	1886,1850(primer blast reference)	100	100	100
		6	Hd1 AGC	65	441 (BL1Sakha101)- moderate heading, 490 (Giza171) -late heading, 620 (Giza177, Sakha 103) - early heading date	140	140	140
	Hd3a	6	Hd3a	62	1108,2163(primer blast reference)	90	90	90
	Hd5/DTH8	8	DTH8-INDEL	57	121 (primer blast reference)	480	70	140
Seed Micronutrient Content	YSL2	2	vf0226164188	62	137	137	200, 300	137
			vf0226169288	62	300	300	NA	NA
			vf0226164382	65	157	157	NA	NA
	YSL15	2	02g43410	55	486	486	NA	310
	YSL13	4	04g44300	55	223	223	NA	NA

Standardization Of Selected Markers With Peanut Genotypes

The 41 markers that showed amplification with rice were standardized with both wild groundnut genotypes *(Arachis glabrata*and *Arachis villosa*) and with three cultivated groundnut varieties *viz.*, Dharani, TPT 3 and ICGV 00350 initially, to observe the amplification in peanut (**Supplementary Fig. 2**). Out of 41 amplified rice markers, 31 (76%) showed amplification with cultivated groundnut genotypes, except sd1-h marker, that amplified only with wild groundnut genotypes. However, 25 showed amplification with diploid wild groundnut except RM3328, GW8PRO2, RM505, RM16942 and YSL15 markers, which are amplified with cultivated groundnut (Table 3).A total of ten markers viz., s5-803, s5-1, HY2-4, Dep1indel1, Dep1-1, SR17, RM593, GW004, YSL13 and YSL2 showed amplification, only with rice.

Table 3
Overview of the markers used in the study and their amplification status

Crop	Rice yield markers		Groundnut oil quality markers		Mungbean Bruchid tolerance		Total markers		% Amplification across genomes
	Markers screened (genes targeted)	Marker amplified	Markers screened (genes targeted)	Markers amplified	Markers screened (gene number)	Amplification status	Markers used	Amplified	
rice	45 (24)	41 (91%)	4(2)	3 (75%)	1(1)	Not amplified	50	44	88
Ground nut	41	31 (76%)	4	4 (100%)	1	1	46	36	78

Cross Transferability Of Markers Between Rice And Peanut

During the course of crop evolution, the genome content across plant kingdom is proved to be conserved and is evident from many synteny studies across genera/families. Further, conservation of functional genes and important motifs are reported by many earlier research groups (Trivedi et al., 2013; Hussien et al., 2014). The transferability was analysed with similar allele sizes of groundnut genotypes to rice *i.e.* which are observed across the genotypes under study.

Plant Height

Plant height is screened with *sd1-h* marker of*sd1*gene, which is responsible for dwarf stature of the plant that encodes gibberellin 20-oxidase (*GA20ox-2*). This gibberellin 20-oxidasehelps to prevent severe dwarfism in *sd-1*mutants. Habataki rice variety expressed 843bp of allele size for semi-dwarfism, whereas Sashaniki expressed 800bp size for tallness in rice. In the current study, this marker showed amplification of 843bp in rice only (as in Monna et al., 2002) and 190bp product size in wild peanut, whereas cultivated peanut didn’t show any amplification. Thus, it might be due to the massive changes happened at the primer locus or may be due to the complete loss of respective alleles/gene while evolution.

Plant Architecture

OsSPL14 (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 14), also known as WEALTHY FARMER'S PANICLE/IDEAL PLANT ARCHITECTURE 1 is regulated by a microRNA, OsmiR156. Increase in level of transcript of *OsSPL14* increases grain productivity and also ideal plant architecture (*IPA*) phenotype (Miura et al., 2010) in rice. This marker amplified 500bp allele size in 11 groundnut genotypes *i.e.* similar to rice allele, **(**Fig. 2**)** except in Narayani, Kalahasti, Prasuna, Rohini, Bheema, Dharani and Nithya Haritha, which amplified 70bp allele size. However, Greeshma showed 390bp allele upon amplification. *OsSPL14* functions for heavy panicle with lot of secondary branches and also it promotes ideal plant type in rice(Mohapatra et al., 2018). In groundnut secondary branch number is a major yield governing trait. Thus, characterizing of this gene in peanut might help in improving yield potential by altering the branching and flowering/pod formation pattern.

PLANT ARCHITECTURE AND YIELD 1 (*PAY1*) gene improves plant architecture and grain yield. The tagged marker, PAY1SP6 was used to identify the transferability from rice to peanut. Interestingly, it was observed an allele of 200bp in rice and both wild and cultivated peanut genotypes (Fig. 2**).** Zhao et al. (2015) in their study, selected a wildrice introgression line YIL55, which displays short plant height, high tillering, thin stems, fewer grains and low yield and a mutant with modified plant architecture called *PAY1*. When compared, *PAY1* exhibited greater plant height, lower tiller number, smaller tiller angle, thicker stems and larger panicles due to the presence of longer internodes, more vascular bundles in stems, and production of more secondary branches. NILs in Teqing or 9311 genetic background also demonstrated that *PAY1* could shape better plant architecture and enhance grain yield of rice which reveals that PAY1 is a dominant regulator of plant architecture. Hence, characterization of these kinds of genes can help better in shaping the groundnut pant along with production of more number of reproductive units.

Yield Contributing Genes

Grain number (GN2 **)** which functions as *OsWAK* (Wall-Associated Kinase) receptor like-protein is responsible for increase in grain number. The marker, RM3535 (Rice Microsatellite) which is closely tagged to *GN2* gene was selected for study. This marker amplified 185bp in all the genotypes of peanut which is in accordance with rice. Sequencing of this allele can further strengthen its conformity and to use the allelic variants in groundnut improvement breeding **(**Fig. 2**).**

The YLD **(yield) gene** linked SSR marker RM223 amplified 165bp in all genotypes of rice and peanut (Fig. 2). This marker also has tagged to aromatic/non-aromatic trait of rice as reported in Jewel et al. (2011).

SPIKELET NUMBER (SPIKE **)** is allelic to Narrow Leaf1 (*NAL1*) that encodes a plant-specific protein with an unknown biochemical function. SPIKE-INDEL3 marker of *SPIKE* gene amplified 171bp in rice, which can increase spikelet number, as reported by Kim*etal.*(2016). In groundnut also the 171bp allele was observed *i.e.* similar to rice. However, the marker showed non-specific amplification of 250bp fragment along with 171bp among cultivated peanut genotypes (Fig. 2).**Erect panicle 3 (**EP3**)** encodes a putative F-box protein and is associated with erect panicle. S5-803 marker expressed 243bp allele size only in rice as described in Piao et al., 2009 (Supplementary Fig. 1).

SCM2/ABERRANT PANICLE ORGANIZATION 1 (APO1 **)** encodes an F-box-containing protein which is involved in controlling rachis branching in panicle, tiller outgrowth, and culm diameter. *SCM2* (STRONG CULM2) is a mild allele of *APO1* found in Habataki variety and showed increased culm diameter and grain number per panicle without a reduction in tiller number. This *SCM2*-Habataki allele is regarded as a useful allele of *APO1* gene for increasing yield and lodging resistance in breeding programs(Kim et al., 2016). In the current study, SCM2 INDEL1 marker amplified at 117bp in all genotypes of rice and peanut. However, the amplification intensity in peanut is low compared to rice. This can be implied to the presence of similar gene in groundnut with changes happened at the primer annealing site. Therefore, designing of primers for other parts of the gene can justify the presence of *SCM2* ortholog in groundnut, strongly.

DENSE AND ERECT PANICLE 1(DEP1 **)** which encodes phosphatidyl ethanolamine binding protien (PEBP) was selected which is responsible for three different traits such as dense panicle, high grain number per panicle and erect panicle. Gain of function mutation in *DEP1* showed increased number of primary and secondary branches and also grain number per panicle. *DEP1* also controls number of panicle branches through cytokinins because expression level of *OsCKX2*'s down-regulation (Huang et al., 2009). Four markers *viz*., exDEP1, DEP1INDEL1, Dep1s7, DEP1-1 were used for genotyping analysis of groundnut. Of these markers, exDEP1 didn't show amplification with any genotypes. DEP1INDEL1 and DEP1-1 produced amplicons of 1030bp (as in Kim et al., 2016) and 1860bp which indicates non-erect panicle type (as in Li et al., 2017), respectively in only rice genotypes (**Supplementary Fig. 4**) and no amplification was seen in peanut genotypes. The marker Dep1s7 expressed 127bp allele size in rice and all peanut genotypes *i.e.* both wild and cultivated by which study of this gene ortholog is possible from rice to peanut in order to identify pod number increase per plant(Fig. 2**).**

Seed Quality Genes (Seed Size And Weight)

Seed size genes of rice were utilized to found orthologs in other cereals. Thus the current study proceeded to found peanut orthologs with the following seed size governing genes of rice.

GS2 **(Grain Size)** is involved in the regulation of grain length and width in rice. It also functions as dominant regulator for grain shape. The marker RM3212 located on chromosome 2 was associated with medium-grain phenotype (Zhang *et al*.,2013). This marker expressed 181bp of allele size in rice and peanut.

GS3 **(Grain Size)** linked markers are RGS1, SF28, RGS2 and SR17. *GS3* was not detected in African cultivars of rice (*O.glaberrima* and *O. barthii*) due to domestication process whereas the wild relative of rice *O. meridionalis* has unique alleles with respect to *GS3* and shares the genome of AA with *O.sativa* which represent good candidate gene for genetic improvement of cultivated lines. Allelic variations at three loci including SF28, RGS1, and RGS2 in GS3 were highly associated with grain length, and explained a large portion of the variations in the mini-core collection of Chinese rice germplasm. SR17 has marginal effect to grain length (Wang *et al*.,2010).RGS1 is well predictive of medium to short grain length in rice. In the present study it amplified 180bp and 200bp allele sizes. SR17 amplified 1400bp of allele size, only in rice (**Supplementary Fig. 4**). These two markers regulate grain size as well as grain length in rice.

GS5 **(Grain Size)** encodes putative serine carboxypeptidase and regulates grain width positively. Overexpression of *GS5* results in increased grain width (Li et al., 2011).Four markers RM574, RM593, C62, GS5-INDEL1 were used for genotyping analysis. Of these, C62 marker didn't show any amplification. RM593 marker amplified with a size of 279 bp only in rice(**Supplementary Fig. 4**).GS5-INDEL1 amplified an allele with a size of 67bp in all the genotypes of rice and peanut which suggests that transferability of marker can be possible from rice to peanut. This 67bp allele size indicates wide grain(ST6) size as reported in Kim et al., 2016(Fig. 3). Hence, identification of functional variation in this gene might help in tagging of seed size in groundnut.

RM574 which is closely tagged to *GS5* locus expressed 240bp of allele size in all genotypes of both rice and peanut **(**Fig. 3). This allele is usually associated with low grain width/size and can be used for rejecting at seedling stage in marker assisted breeding for high grain width character (Bidanchiet al., 2018) in rice. Characterizing of this gene in groundnut would reveal kernel size governing allelic variants.

GW2 **(Grain Width and Weight)** encodes a Ring type E3 ubiquitin ligase which functions in protein degradation pathway. This enzyme negatively regulates cell division and *GW2* mutant allele promotes spikelet hull cell division which results in increase of grain width and grain weight (Song et al., 2007). GW2SNP2 marker expressed 51bp of allele size in both rice and peanut genotypes (Fig. 3) as reported same allele size in Zhang et al., 2015, by showing Kasalath with 51bp allele size whereas TD70 showed 30 and 21bp fragment after digestion with *ApoI*. The STS marker GW004 was used for amplification of *GW2* gene which enhances grain width and yield. This marker reported the alleles of 750bp and 1050bp (Ngangkhamet al., 2018) in rice. In our study this marker expressed 750bp only in rice, which denotes lower grain width but not in groundnut (**Supplementary Fig. 4**).

GW5 **(Grain Width and Weight)** is associated with grain width or seed width. RM3328 which is linked to *GW5*at 2.3cM was selected for analysis of *GW5* gene region. This expressed 119bp product size in rice (as same in Wang et al., 2008) and 95bp allele size in cultivated peanut genotypes whereas wild groundnut didn't show any allele (**Supplementary Fig. 3**). The gene couldn't reveal similar allele pattern, as there might be presence of indels in the region. The sequence analysis of the allele can show the possible reason behind allele change or entirely off-target by the marker.

Another marker RMw513, which is closely linked (0.37cM) to *GW5* was selected for molecular analysis. This marker expressed 600bp allele size in both rice and cultivated peanut genotypes along with additional lower size alleles. The wild peanut showed 700bp of allele size (Fig. 3). RMw513 marker is associated with multiple traits such as grain width (GW), length- width ratio (LWR) and degree of endosperm chalkiness (DEC). RMw513 is used in MAS for developing slender grain and low chalkiness (Zhao *et al*.,2015) in rice. Analysis of the *GW5* ortholog in peanut might help to unravel the seed size variation.

***SW5* (Seed Width)** N1212 marker linked to *SW5* is associated with increased grain width. In the research conducted by Shomuraet al. (2008) between Nipponbare and Kasalath, Nipponbareharbored a 1212bp deletion and this deletion is Functional Nucleotide Polymorphism (FNP) for *qSW5*. This marker expressed 759bp product size in case of Nipponbare compared to 1971bp in Kasalath (Zhang et al., 2015). But in our study this marker expressed 65 bp product as major allele in all genotypes of rice and peanut (Fig. 3).

Single major QTL (qsgw7) was identified on short arm of chromosome 7which deals with 1000-grain weight. Grain weight is directly associated with increased yield. The markers RM22015 and RM21997 which are closely linked to this gene were determined by Bian et al., 2013. Amplification with RM22015 marker amplified 176bp of allele size only in rice genotype in the study but not in groundnut (**Supplementary Fig. 4**)

GLW7 **(Grain Length and Width)** encodes plant specific transcription factor OsSPL13, which helps in regulating cell size in grain hull that inturn enhances grain length and yield. This belongs to *SQUAMOSA PROMOTER BINDING PROTEIN (SBP)* family and plays an important role in plant growth and development. *OsSPL13* has a critical factor in the divergence of tropical *japonica* and temperate *japonica*, providing the opportunity to improve grain size and grain yield for a majority of temperate *japonica* varieties by introducing the large-grain *OsSPL13*LGH allele when breeding new elite rice varieties (Si et al., 2016). This marker expressed 140bp size in both rice and peanut. However, some genotypes of cultivated peanut *viz.*, TPT-4, Kalahasti, Abhaya, TCGS894,TCGS1157 showed 160bp of allele size(**Supplementary Fig. 3**).This might give an assumption that variations occurred at the locus among groundnut genotypes. In-depth study of the gene will facilitate thorough understanding of its function in peanut.

RM505 linked to qgrl7 responsible for grain length was selected for genotype analysis. This marker amplified 220bp in Sonasal (short grain), 170bp in Chiguhong (grain plumpiness) and 180bp in PB1121(extra-long slender grain)(Deepti *et al.*,2012;Liu *et al.*,2017). In our study this marker expressed 500 and 180bp in rice and 490bp in cultivated peanut genotypes whereas no amplification is seen in wild peanut and TPT 1 **(**Fig. 3**).** Apart from major allele, non-specific amplification observed in peanut at low intensity. However, sequencing of the major allele can establish the ortholog nature of the gene in groundnut.

The other marker RM21945 present on chromosome 7 responsible for grain length and also quality parameter like Gel Consistency was selected and further analysed. This marker expressed 292bp of allele size for the trait of Gel Consistency. This marker is also associated with grain weight (GW), length and width ratio (LWR) (Zhao *et al.*,2015; Verma *et al.*,2015). In our study also, this marker amplified 292bp in all the genotypes of rice and peanut but with low intensity in peanut, as the reasons explained earlier (**Supplementary Fig. 3**). These results can suggest us that transferability is possible from rice to peanut once analysed with additional set of markers.

Seed Filling

Shelling percentage is an important trait to be considered in groundnut. The rice GRAIN INCOMPLETE FILLING 1 (*GIF1*) gene was considered to identify the ortholog in groundnut. *GIF1* of rice encodes cell wall invertase and is responsible for grain filling and grain weight in rice. Mutation in *GIF1* results in slower grain-filling which inturn reduces the glucose, fructose and sucrose levels in *gif1* mutants (Wang et al., 2008). This marker expressed96bp of allele size in all the genotypes of both rice and peanut (Greeshma with low intensity) **(**Fig. 3**)**, which suggests that transferability of gene that might govern seed filling/size can be possible.The other marker which is closely linked to *GIF1* was RM16942. This marker expressed 181bp of product size only in rice but not in peanut.

Flowering Time

Groundnut is majorly a rainfed crop in India. Hence, early flowering and short breeding cycle is desirable trait among farmers. Heading date or Flowering time determines the beginning of the reproductive cycle and is greatly affected by environmental conditions (e.g. day length and temperature. HEADING DATE 3a (*Hd3a*) and RICE FLOWERING LOCUS T (*RFT1*) are homologs of FLOWERING LOCUS T (*FT*) in *Arabidopsis* (Takahashi and Shimamoto, 2011) and act as florigen genes to accelerate flowering (Ye et al., 2018). *Hd3a* promotes heading under short day conditions, whereas *RFT1* is a major floral activator under long day conditions. *Hd1*, a homolog of *CONSTANS* in *Arabidopsis*, promotes flowering under short day conditions and reduces flowering under long day conditions by regulating the expression of *Hd3a* (Yano *et al.*, 2000). Similarly rice *Hd1* to wheat- *TaHd1* (Nemotoet al., 2003) was identified.These results will boost the assumption of identification of peanut orthologs to rice. In our study, different markers related to heading date *viz.*, Hd1, Hd1AGC,Hd3a and DTH8-INDEL were used.

Hd1 amplified 100 bp of allele size, only in rice. But these were not similar to reported size. The markers,Hd1AGC and Hd3a amplified 140bp and 90 bp respectively, in all the genotypes of rice and peanut with less intensity. Same size of allele can lead to the assumption of the presence of gene similar to rice. Sequencing of the alleles will confirm the assumption. Further, designing and use of additional markers targeting the gene can overcome the problem(**Supplementary Fig. 3**). Molecular characterization of these kinds of genes can help in designing the varieties with preferred duration.

Seed Micronutrient Content

Fe and Zn are important micronutrients towards human health, thus needs to be focused in research of food crops. For Fe and Zn content, primers using primer 3 software were designed for 3 reported genes *viz.*, *YSL2*, *YSL13* and *YSL15* which belongs to Yellow-Stripe Like gene family. *YSL2* gene reported for preferential expression in the leaf tissues which suggests that this *YSL2* functions as transporter which is responsible for the phloem transport of iron. The other gene *YSL15*proved for its significant expression in root and rhizome type of tissues indicating its role in the uptake/absorption of iron from the source (Menna et al., 2011). Out of the five *indel* markers used, only one marker (vf0226164188) tagged to *YSL2*gene amplified alleles (137bp) in both rice and peanut **(**Fig. 3**) and** (**Supplementary Fig. 4**).

Assessment Of Cross Transferability Across Genotypes

From the results, overall transferability of markers under study and their possible utilization in peanut was assessed and are presented in Fig. 4 **and** Fig. 5. It is very interesting to note that amplification of 76% (31 out of 41 amplified markers) of rice gene tagged markers in groundnut was observed in initial standardization. This can be implied to highly conserved nature of functionally characterized genes between rice and peanut.

The extant of amplification of rice gene tagged markers among the peanut genotypes was assessed at individual genotype level from the current study, and allele code was assigned for each genotype (Table 4). The common allele sharing among the groundnut genotypes with rice was ranged from 79.17% (TCGS1157-Nithya Haritha, Greeshma, Prasuna, Kalahasti, Narayani and wild genotype *A. villosa*) to 91.67% (TCGS 1073 -Dheeraj) (Fig. 4). This further confirms the highest possibility of use of the rice markers/genes in groundnut.

Table 5
Total number of rice yield and seed quality gene orthologs found across *Arachis hypogaea* genome and their chromosome distribution

	Rice gene orthologs of *A. hypogaea*								
Chr	***GW2***	***GW5***	***GW8***	***GS2***	***Sd1***	***HD3A***	***Gn1a***	***OsSPL14***	**Gene number per chromosome**
Chr 1	2	2	0	0	0	0	5	0	9
Chr 2	0	0	0	0	2	5	0	0	7
Chr 3	0	4	0	0	2	1	2	0	9
Chr 4	2	1	0	0	1	3	1	0	8
Chr 5	1	1	0	0	2	2	0	0	6
Chr 6	2	4	0	1	0	3	2	0	12
Chr 7	0	0	0	0	0	0	0	0	0
Chr 8	1	0	0	0	1	2	2	0	6
Chr 9	1	0	0	0	2	0	1	0	4
Chr 10	2	1	0	0	1	2	2	1	9
Chr 11	2	3	0	0	0	0	6	0	11
Chr 12	1	2	0	0	1	3	0	0	7
Chr 13	1	2	1	0	3	1	2	0	10
Chr 14	1	0	0	0	1	3	0	0	5
Chr 15	0	1	0	0	2	2	0	0	5
Chr 16	1	6	0	1	2	3	2	1	16
Chr 17	0	0	0	0	1	1	2	0	4
Chr 18	0	0	0	0	0	1	0	0	1
Chr 19	1	0	0	0	2	1	0	0	4
Chr 20	2	1	0	0	0	2	1	1	7
Total	**20**	**28**	**1**	**2**	**23**	**35**	**28**	**3**	**140**

Further the transferable efficiency of individual marker revealed that a total of 17 markers pertaining to 14 genes (Fig. 5) showed amplification among all groundnut genotypes (100%). However, two markers Dep1 INDEL 1 and Hd1 showed 6% amplification only. Of 17 markers, few of which (SCM2INDEL, Hd3A, Hd1AGC) showed the amplification at low intensity yet retained the allele size of rice. Hence, designing/use of markers from rest of the gene sequence would resolve the amplification problem.

Insilico **Analysis**

Insilico analysis of groundnut genome with the help of Peanutbase(https://peanutbase.org/search/gene) and NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed large number of rice yield gene orthologs. From the Peanutbase, the analysis has identified 140 rice gene orthologs of *Arachishypogaea* for different traits (Fig. 6). Of which, eight grain size related genes viz., *HD3A*(35 genes), *GW5*(28 genes), *Gn1a*(28 genes), *sd1*(23 genes), *GW2*(20 genes), *OsSPL14*(03 genes), *GS2*(02 genes) and *GW8*(01 gene) genes were identified, with greater number (79)of peanut orthologs i.e. 56.43%.Remaining genes are found with plant architecture governing genes.The gene distribution on different peanut chromosomes is shown in the Fig. 6. The total of 140 genes fell on all chromosomes except Chr 7. Chromosome 16 contains highest number (16) of orthologous genes followed by Chr 6 with 12 genes. Only one orthologous gene was identified for GW8 on chromosome 18. Physical location of genes was given in **Supplementary Table 3**.

By *Insilico* analysis, we identified 20 Ubiquitin ligase genes (GW2 orthologs) located on 14 different chromosomes (Chr 1,4,5,6,8,9,10,11,12,13,14,16,19 and 20) in peanut pertaining to Grain weight/seed weight (Supplementary Table 3). These functionalized genes are reported to increase cell number and also regulate cell division. Characterizing these genes will help to regulate seed weight in peanut. Recently Wang et al. (2021) also proved similar kind of results with peanut seed transcriptome study.

Primer blast analysis of NCBI database with rice gene tagged primers under study, for expressed sequences/transcripts of groundnut genome also revealed the presence of huge number of functional rice yield gene orthologs for diverse traits(**Fig. 7**).

Studies have proved the transfer of knowledge of functionally characterized genes of Rice (poaceae) to other plant families. For instance, grain size (*GS2*) of rice to fruit size (*OVATE*) of Tomato (Gupta et al., 2006.), tillering of rice (MOC1) to Lateral suppressor (Ls) gene of tomato (Hussienet al., 2014).

The studies on utilization of homolog genes proved the conservation of coding sequences between rice and other cereals/millets. For instance, study on identification and utilization of rice grain width and weight gene (GW2) homologs in maize revealed the presence of two genes *viz.*, *ZmGW2* on CHR4 and *ZmGW2* on CHR5 (Li et al., 2010). Sequence analyses of these genes showed highly conservedcoding regionwith an overall similarity of 94% with rice wherein the functional protein domains of both genes are completely conserved, with no non-synonymous polymorphisms identified, which suggests that both genes have conserved functions across genera.

Nemotoet al. (2003) also isolated three kinds of *Hd1* orthologs from wheat *TaHd1-1, TaHd1-2, TaHd1-3* derived from A,B and D genomes respectively. When they introduced *TaHd1-1* into *Hd1* deficient rice line, transgenic plants complemented the functions of *Hd1* promoting heading under short day conditions and delayed under long day conditions.Further, the research on *Hd1* of rice found that *Hd1* is a homolog of *CONSTANS* (*CO*)gene of*Arabidopsis* and encodes a protein with a zinc finger domain. Sequence comparison of *Hd1* with that of *CO* from *Arabidopsis* found 59% identity in the zinc finger domain and 79% identity in the C-terminal region.

Collinearity of cereal and legume genomes analysed with genomic sequences of molecular model crops also proved to have similarity of over 40% (**Supplementary Table 4**).Interestingly, Hussienet al., 2014 characterized Rice-nodulation signaling pathway genes *NSP1* and *NSP2*and identified the orthologs in Legumes *viz.*, Legume-NSP1 and Legume-NSP2.This study proved the transferability of known gene knowledge from rice to legumes.

Conclusions

The progress in functional characterization of genes for desired traits in groundnut is very slow and success is meagre. This is because of complex genome nature of groundnut (Burrow et al., 2001; Janilaet al., 2016) and also due to the availability of low diversity at molecular level.

The results of current study that showed high transferability of gene tagged markers, due to conserved nature of functional genes between molecular model crop rice and peanut, are encouraging to characterize these genes in groundnut.

Insilico analysis also identified huge list of rice orthologs for different traits. Hence, characterizing this repertoire of genes both with forward and reverse genetic approaches would help in rapid tagging of peanut genes. Sequencing approaches are more powerful to reveal the *indels* and thereby the functional variants at respective genes rather analyzing with conventional methods, especially in complex genomes like groundnut. Genome Wide Association mapping approaches (GWAS) are also very useful in tagging the genes/gene families for desired traits.

In brief, utilization of the available knowledge of functionally characterized genes of molecular model crops and vast list of annotated orthologous genes present in 'Omics' databases, widens the scope to improve the orphan crops with low molecular progress like peanut by pyramiding of desirable genes in short span of time by the breeders.

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Author Contributions

All authors contributed to the study conception, design, manuscript preparation etc

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Availability of Data and Materials

The data presented in this article were taken from NCBI blast and also Peanutbase.

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Tables

Table 4 is available in the Supplementary Files section.

Figures

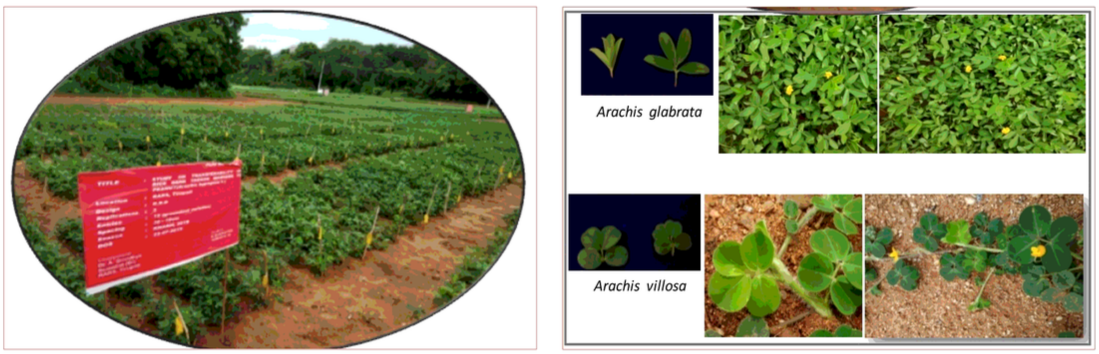

Figure 1

Field view and wild genotypes used in the study

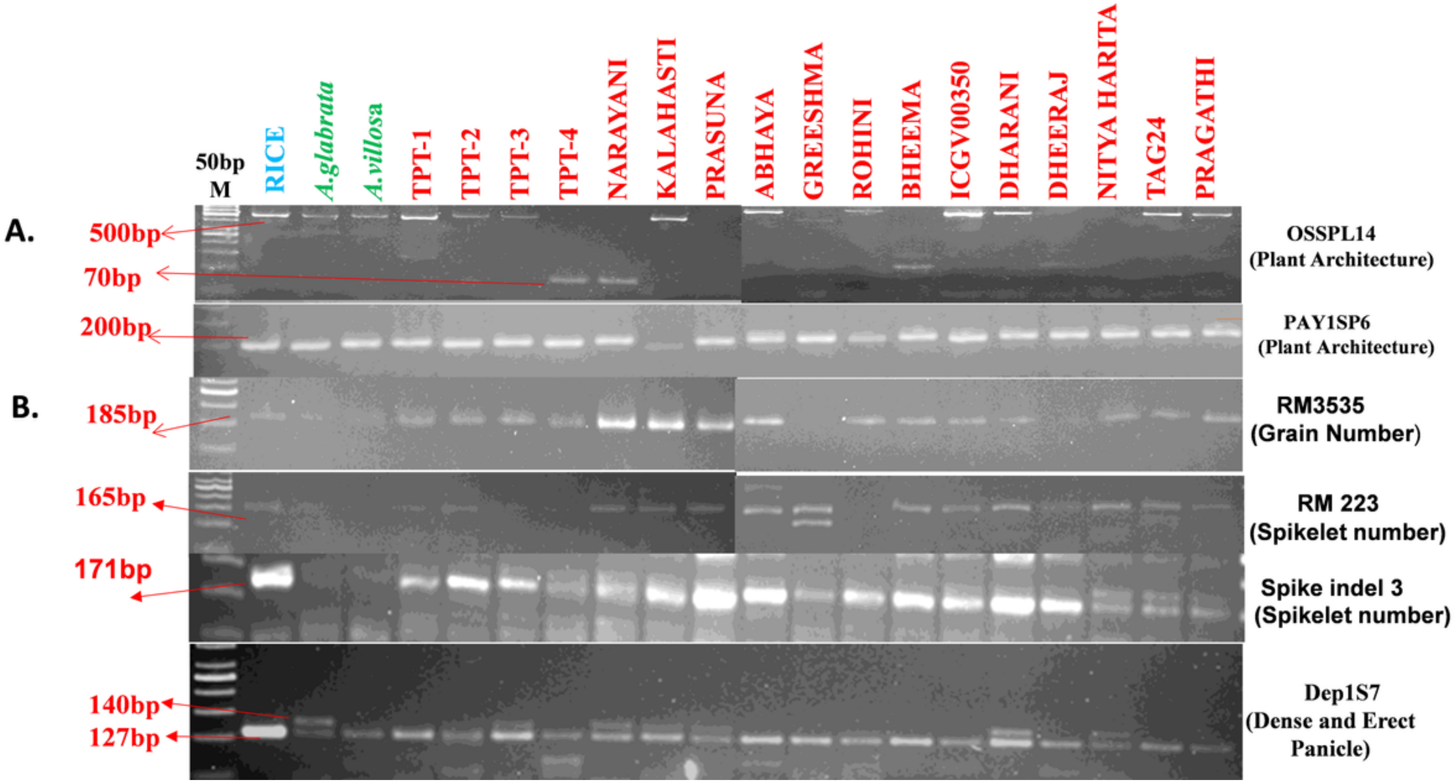

Figure 2

Amplification of Groundnut genotypes with rice Plant Architecture (A) and Yield gene tagged markers (B)

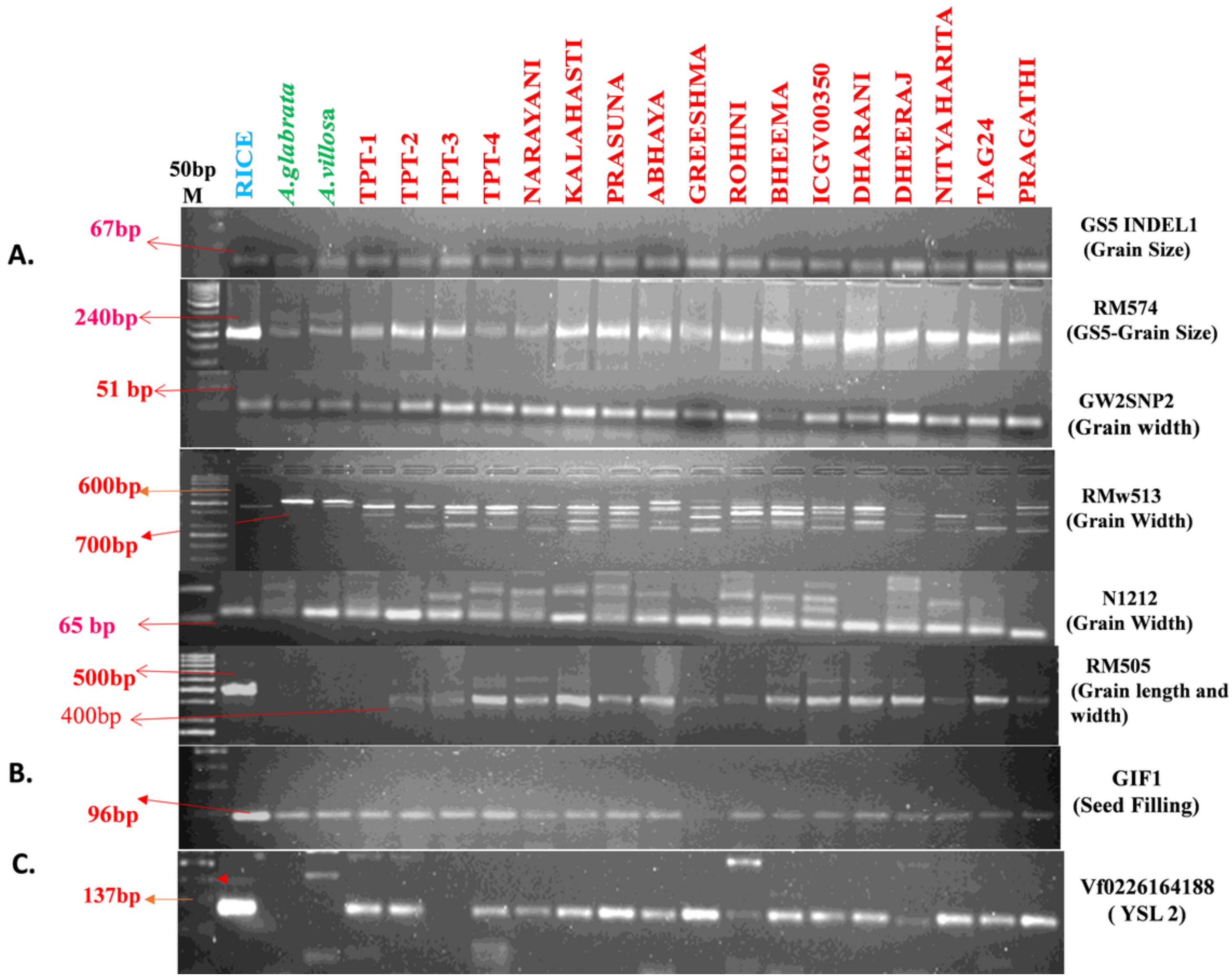

Figure 3

Amplification pattern of Groundnut genotypes with rice Seed quality (A), Seed Filling (B) and Seed Micronutrient (Fe and Zn, Yellow Stripe Like 2 -YSL 2) (C) Gene tagged markers

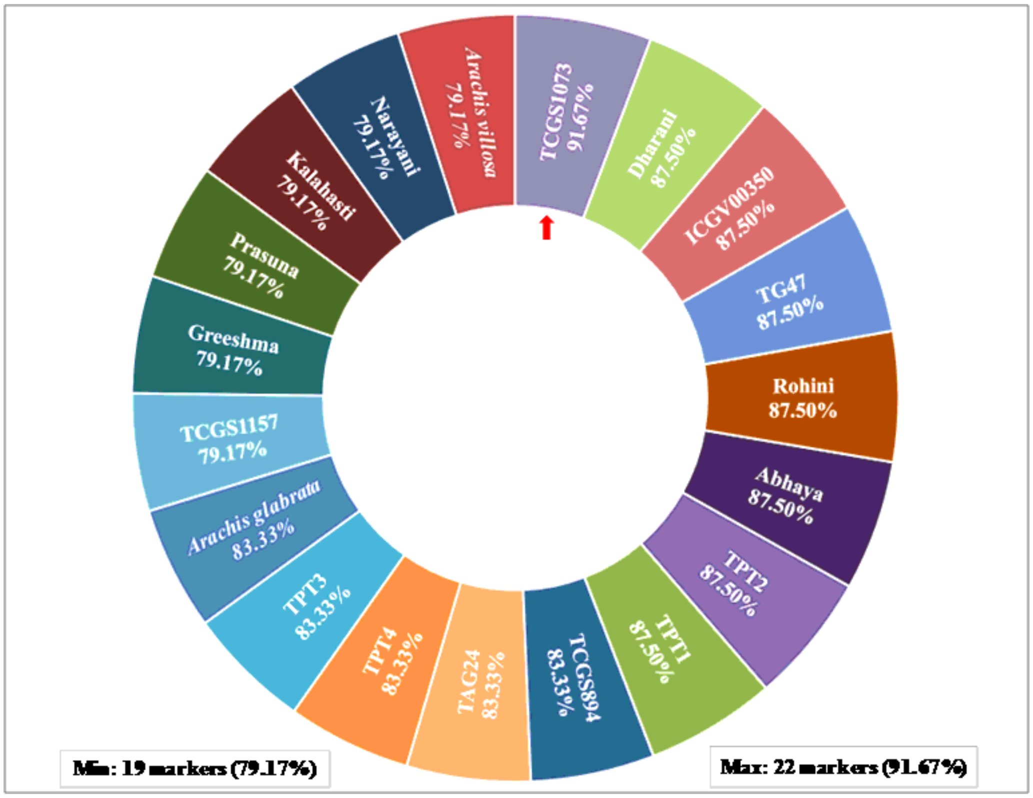

Figure 4

Comparison of individual groundnut genotypes for amplification status (%) with reported rice gene tagged markers

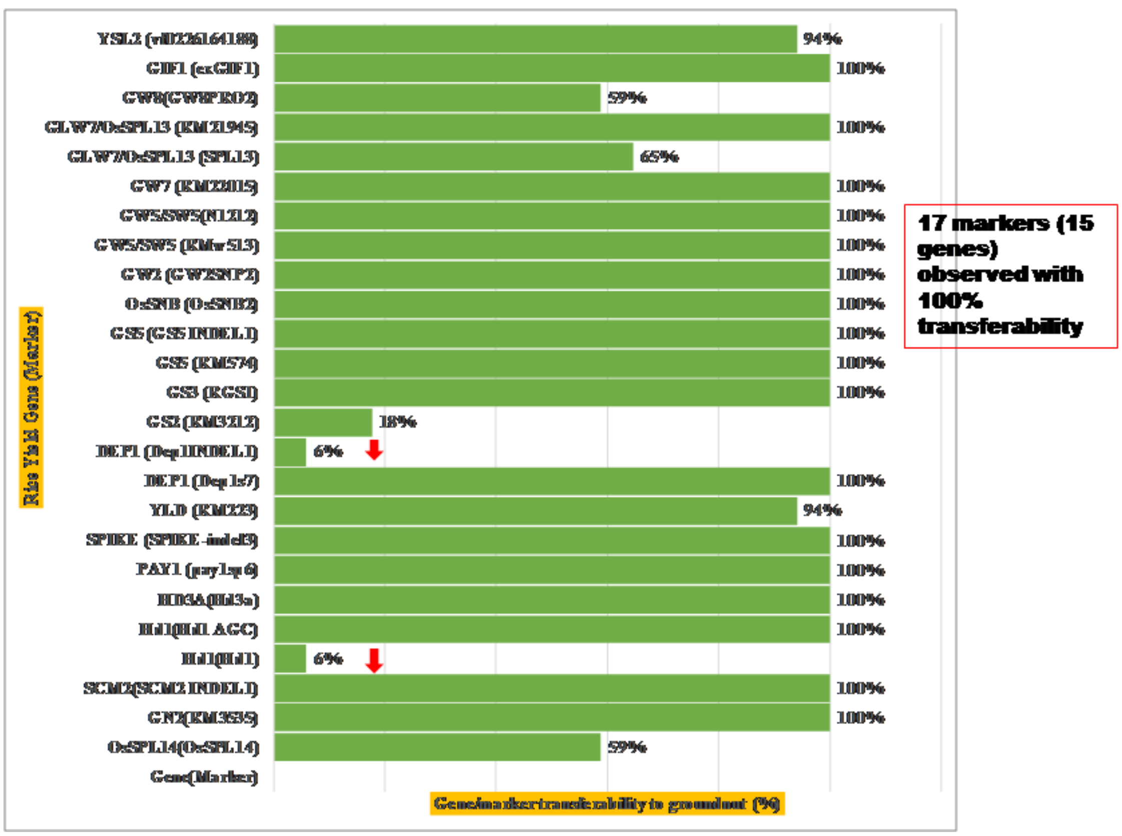

Figure 5

Estimation of transferable efficiency of rice gene targeted markers to groundnut and possibility of presence of rice ortholog genes

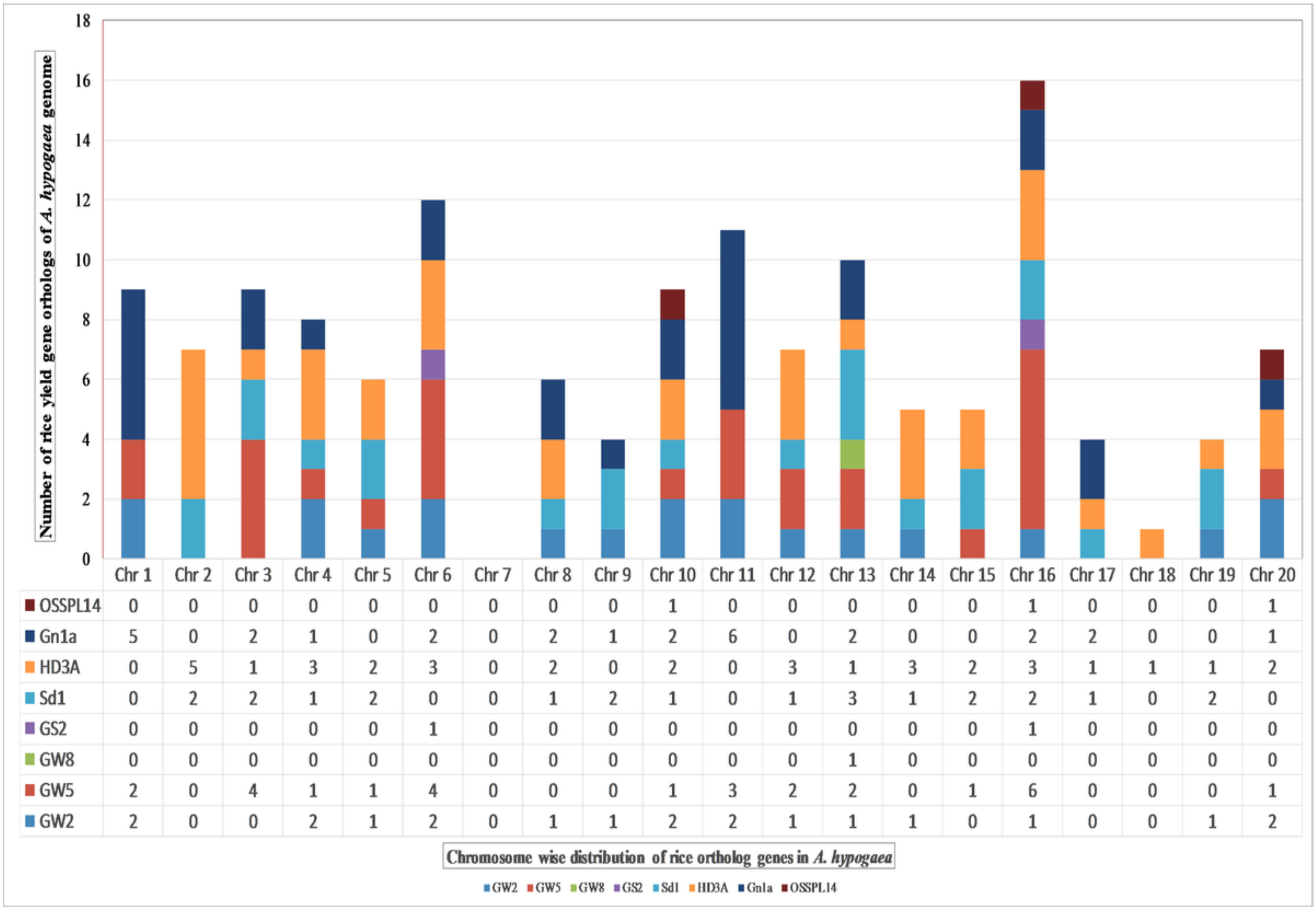

Figure 6

Chromosome wise distribution of rice ortholog genes in *A. hypogaea*

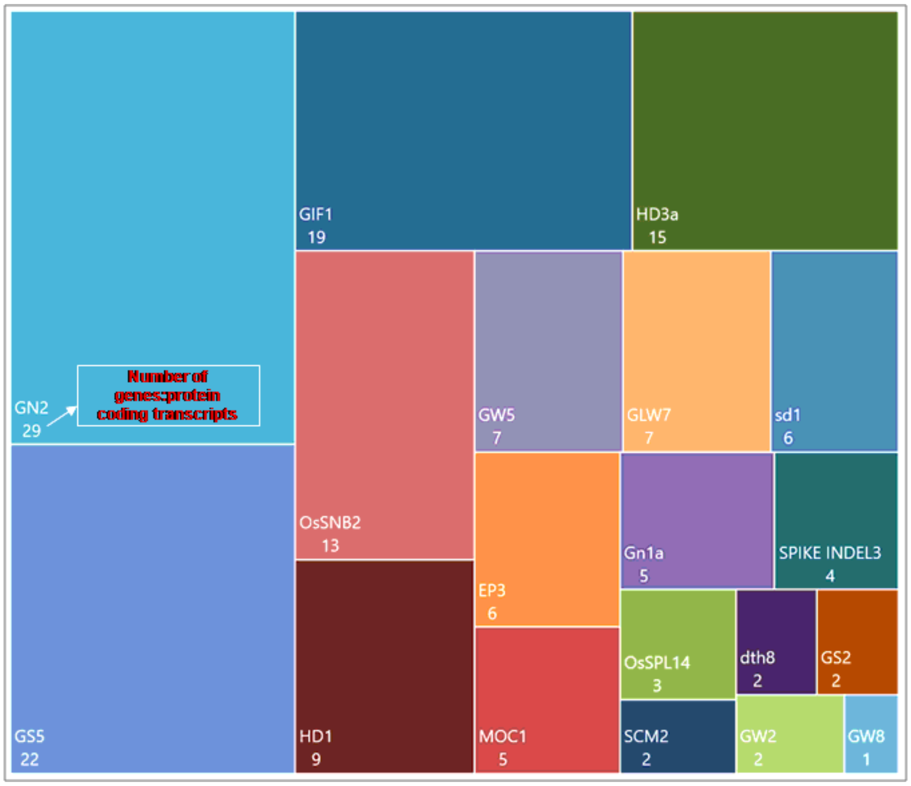

Figure 7

Tree chart showing *A. hypogaea* transcripts orthologous to rice yield genes

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation1.docx
- TABLE4.docx