Original Research Article

Variation in Crude Protein Content Among Recombinant Inbred Lines of Rice (Oryza sativa L.)

Abstract

Cereals constitute the primary dietary staple for humans, supplying a significant portion of energy, protein, and essential nutrients. Protein, crucial for growth, antibody production, and immunity, ranks as the second most abundant storage component in rice grains. Various factors, including grain protein content, amino acid composition, and fat content, influence the nutritional quality of rice, with grain protein content being particularly significant. This study conducted screening and evaluation of crude protein content across 200 recombinant inbred lines of rice, derived from an inter-specific cross between BPT5204 and HPR14 parents. Results indicated a range of total crude protein content from 13.17 (mg/g) to 28.94 (mg/g) among samples. Grouping analysis based on available crude protein content categorized the lines into three major groups: approximately 18% (36 lines) exhibited significantly higher protein content (>25mg/g), 69% (140 lines) displayed moderate content (15 mg/g to 25 mg/g), and 13% (26 lines) showed low protein content (<15 mg/g). The identification of high protein content genotypes in this study holds potential for mapping key genomic regions associated with protein content in rice.

Key words: Rice, crude protein, RILs.

Introduction:-

Rice stands out among cereals as a fundamental dietary staple for over half of the global population, profoundly shaping the cultures, diets, and economies of billions, particularly in Asia with its extensive history of cultivation deeply integrated into daily life (Narayanan et al., 2000). However, cereal-based diets have contributed to widespread undernutrition, especially protein deficiency, a pressing issue in developing nations where only twenty percent of the population has access to a nutritious diet. Protein energy malnutrition affects approximately 230 million children in these countries (WHO Bulletin, 1993), with over 50 percent of Indian children receiving insufficient calories daily for optimal growth (Mahendra et al., 2004). Sub-Saharan Africa and South Asia remain focal points for child malnutrition (Andersen et al., 1999). Studies on children recovering from protein energy malnutrition highlight the importance of essential amino acids like lysine and tryptophan in enhancing nitrogen retention (Pellett and Ghosh, 2004). The milling process of rice grains, while removing the bran layer, not only eliminates a nutritionally valuable component but also reduces the quantity of rice available for human nutrition by up to 15 percent. Glutelin constitutes the majority of seed protein, with albumin and globulin major components in rice bran (Cagampang et al., 1966; Juliano, 1972; Villareal and Juliano, 1978).

Successive milling layers reveal that the outermost fraction holds the highest protein content (Hogan et al., 1964).Rice provides a significant portion of calories and protein in tropical Asian diets (Shobarani et al., 2006), although it lacks in lysine and tryptophan compared to other protein sources (FAO, 1957). However, rice protein contains the highest lysine content among cereal proteins. Banerjee et al. (2011) observed a positive correlation of tryptophan with lysine content but a negative correlation with total protein content. Environmental factors significantly influence cereal protein content, with rice exhibiting a wide range of grain protein accumulation (IRRI, 1963). Milled rice has relatively low protein content, while brown or dehulled rice contains more protein. Increasing rice protein content could enhance protein supply in rice-based diets.Rice harbors extensive genetic diversity, offering a rich resource for essential nutrients such as zinc, iron, proteins, and vitamins. Screening and exploiting this diversity are critical for genetic improvement aimed at addressing malnutrition (Khush and Virk, 2002). Enriching rice with protein, whether through conventional breeding or marker-assisted introgression, holds promise for sustainable food solutions (Graham et al., 2001). The primary objective of this investigation was to evaluate rice protein content variability in an association mapping panel for subsequent genetic enhancement endeavors.

Material and Methods:-

Plant Materials:

Thematerialswhichwereutilizedinthepresentstudyconsistedof200recombinant inbred lines of rice derived by pedigree method by crossing Samba Mahsuri (BPT-5204) and HPR-14.

Experimental design:

The study was undertaken at during Summer and Kharif seasons of the year 2020 in the experimental plots at A block of College of Agriculture, V C Farm, Mandya, which is located at an altitude of 697 m above mean sea level (MSL) and 76˚50'01.7''E latitude and 12˚34'25.4''N longitudes.Care was taken to raise the crop by adopting package of practice, UASB including regular irrigation to raise a good crop growth for both the seasons. Three samples per accession were evaluatedfortotalsolubleproteincontent.Thedriedseedsweredehuskedusinghandpalmdehusker, ground evenly and sieved up to thetalcum powder size with pestle and mortar.Thepowderedsamplesofunpolishedricewere used for protein analysis using modifiedLowry‟smethod(Lowry *et al*., 1951).

Extraction of Protein

Sample preparation

Powderedsampleswere subjectedtoextractionofproteinby0.1Mphosphatebuffer with pH of 7.4. One gram of samplefrom each entry was macerated with 50 ml ofphosphate buffer using Pestle and Mortar andcentrifuged at 15000 rpm for 15 minutes at 4ºC. Thesupernatantswerecollectedforproteinestimation by discarding the pellet. The abovestepswereperformedforeachentryseparately until a clear extract was obtained.The extract was stored in deep freezer untilfurtheranalysis.

Protocol:

TotalproteinwasestimatedbymodifiedLowry‟smethodgivenbyHartree,1972.Determinationofp roteinconcentrationbyultraviolet absorption depends on the presenceofaromaticaminoacidsintheproteins.Although different proteins will have differentaminoacidcompositionsandthusdifferentmolar absorptivities, this method can be veryaccurate when comparing different solutionsofthe sameprotein(UKassays,2017).Extracted samples of 0.2 ml were taken intotest tube and the volume was made up to 1 mlwith distilled water. To it, 4.5 ml of alkaline$CuSO_4$ reagent was added and incubated atroomtemperaturefor10minutesfollowedby0.5mlofFolin‟sphenol reagent.Thecontentsweremixedwellandtheabsorbancewasmeasuredat650nmafter15minutesina spectrophotometer. From the standard graph,the amount of protein in the given unknownsolutionwas calculated.

Result and Discussion:

Protein energy malnutrition impacts a significant portion, 25%, of children who primarily rely on rice and staple crops with low levels of essential amino acids (Gearing, 2015). In Southeast Asia, rice serves as the primary source of both carbohydrate and protein (Cagampang et al., 1966), albeit with the lowest protein level of 7.3g/100g in brown rice among cereals (Souci, Fuchmann, and Kraut, 1986). With a mean true digestibility of 93 percent compared to highly digestible proteins like egg, milk, cheese, meat, and fish (WHO, 1985), rice plays a crucial dietary role.

The recommended dietary allowance for protein intake stands at 0.8 grams per kilogram of body weight per day (Harvard Men's Health Watch, 2015). Despite the presence of other protein-rich foods such as eggs, meat, milk, fish, and pulses, their affordability varies across different sections of society. Low-income groups often rely heavily on rice-based diets. Protein energy and micronutrient malnutrition are prevalent among rural and impoverished populations. Thus, identifying a viable source of rice protein from the available gene pool is imperative to meet the necessary protein thresholds in their diets.

Total Protein Content

Among 200 RILs of rice taken for the present study, the protein content ranged from 14.99 mg/g in BH-RIL-00623 to 28.11 mg/g in BH-RIL-00317 with a coefficient of variation of 10.058 mg/g (Table 1**).**Seven lines were found to have significantly higher protein content than the grand mean of 20.646 mg/g. About 38 % (76 RILs) of RILs had low protein content (<15.00 mg/g) and 3.0 % (8 RILs) recorded higher protein content of more than 25.00 mg/g of sample. About, 59.00 % (119 RILs) of samples recorded moderate level of protein content with range of 20.00 to 25.00 mg/g, (Fig. 1 and Table 2). It could be realized that in general most of the genotypes categorised under moderate protein content. However, there is a handful of material representing 3.00 % of the RILs with high protein that gives the ray of hope that a significant variation exists among the rice recombinant inbred lines taken for our investigation.

Previous studies by Kennedy and Burlingame (2003), Cao et al. (2009), and Silveira et al. (2010) proposed a classification system for protein contents, categorizing them as high (≥12%), medium (11.9–9%), and low (≤8.9%). In our association mapping panel, we follow a classification scheme based on the range of protein content: high (>10.5g/100g), moderate (9.01-10.5g/100g), and low levels of proteins (<9.0g/100g). Similarly, Heda and Reddy (1984) considered values >10% as high protein content in their analysis of F1 to F3 progenies of six crosses. Research has demonstrated that protein content in 1622 milled rice samples from 24 countries ranged from 4 to 14 percent, with a mean protein content ranging from 6.3 to 9.2 percent and an overall mean of 7.8 percent at 12% moisture (Juliano and Villareal, 1993). Kennedy and Burlingame (2003) analyzed the protein contents of 2,869 rice genotypes (2,674 O. sativa and 195 O. glaberrima) and found a mean of 8.8% for O. sativa, ranging from 4.5 to 15.9%. In their present analysis, comprising O. sativa germplasm, the mean protein content is 8.88%, with the highest protein estimation of 14.70 g/100g, closely aligning with their findings.

The crude protein content of ten Philippine rice cultivars, as evaluated by Riza et al. (2004), exhibited a range from 6.3% (PR-27423-MS6) to 9.1% (PR-31595-PSC101). Chandel et al. (2005) reported a wide variation in protein concentration, ranging from as low as 2.8% to as high as 9.9% in milled rice germplasm lines of Chhattisgarh.

Cao et al. (2009) observed considerable variation in storage protein content (ranging from 7.38% to 15.41%) among Chinese varieties of O. sativa rice. Banerjee et al. (2010) found a wide range of protein content (6.19% to 10.75%, mean 8.07%) in brown rice of 46 genotypes, including cultivated indica and japonica cultivars, germplasm accessions, advanced breeding lines, and wild rice genotypes.

Silveira et al. (2010) reported storage protein contents ranging from 4.4% to 20.2% across 550 accessions in the rice Core Collection of Embrapa, with an average of 10.31%. Mohanty et al. (2011) documented crude protein levels of 16.41% and 15.27% in brown rice of ARC 10063 and ARC 10075 rice accessions, respectively, on a dry weight basis.

According to Totok et al. (2011), genotype x location interaction influenced grain protein content in 10 upland rice genotypes. They identified three genotypes—UNRAM 4E, UNRAM 9E, UNRAM 17E—as possessing stable protein content (>8.0%) in their milled rice using Kjeldahl's method. Additionally, Banerjee et al. (2011) analyzed the protein content of milled grain among 258 landraces of rice belonging to the extra-early group (95 days) maintained at IGKV, Raipur, Chhattisgarh. They found a range from 4.91% to 12.08%, with a mean of 6.63%. Out of the total 258 lines analyzed, 52 exhibited <6.0% protein, 202 landraces had protein levels between 6.0% and 9.0%, while four lines showed >9.0% of grain protein.

They observed that the total free amino acid content was higher in these accessions, and lysine content was positively correlated with grain protein content, contrary to the views of Juliano et al. (1964) and Cagampang et al. (1966). Hanumantrao (2013) estimated protein content in brown rice of 58 rice germplasm lines and varieties using the modified Micro Kjeldahl method. Protein content varied from 6.09% in the variety CGR-436 to 11.2% in Danteshwari.

By utilizing ARC 10075 as a donor, CR Dhan 310 (IET 24780) rice variety was developed with a high protein content of 11% and was rich in threonine and lysine (NRRI Annual Report 2014–2015). Santos et al. (2013) analyzed twenty-nine accessions of the wild rice species Oryza glumaepatula, collected from five Brazilian states, and two commercial cultivars for storage protein profile and amino acid content. Total protein levels ranged from 14.94% (wild genotype BGA14280) to 9.07% (BGA14179). The control cultivars BRS Bonança and Primavera, along with the wild accessions BGA14210, BGA14232, BGA14233, and BGA14179, showed the lowest levels of total protein. Seven accessions possessed high protein content, ranging from 13.98% to 14.94%. The second group had nine accessions with a range of 12.3% to 13.35%. Thus, out of 29 genotypes evaluated, 16 had high total protein content, and 13 had medium protein content.

A scrutiny of the above findings on the estimation of protein content by different workers reveals that the low protein category ranged from 2.8% to 7.38%, while the other extreme ranged from 9.07% to 15.9% in genotypes belonging to O. sativa. The lowest value of 7.54g/100g recorded in this study material is above the range mentioned by earlier workers.

Thepresentstudyofthetotalavailableproteincontentin200recombinant inbred lines of ricegrainsusingmodifiedLowry'smethod.Modified Lowry's method was considered ashighly feasible method to estimate even thenegligible amount of protein and this methodprovidestherealpictureofsolubleprotein level which directly depicts the bio-availablelevel of protein content in the human body. InthisstudyAmong 200 RILs of rice 7 lines were found to record significantly higher protein content, 76 lines had low protein content and 119 lines of RILs recorded moderate level of protein content.

Thepresentknowledgeonthetotalaccumulationofproteininrecombinant inbred lines of rice would be helpful for breeders to enhance thelevelofnutrientaccumulationinrice grains.

Innearfuture,conventionalbreedingalongwithmarkerassistedselectionoradvancedgenomic strategies will give fruitful results toachieve the targeted level of accumulation ofproteinsandotherimportantnutrientsingrains.

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Tables:

Table1: Total available crude protein content (mg/gram) in 200 recombinant inbred lines of rice

Sl. No.	RIL No.	PC (mg/g)	Sl. No.	RIL No.	PC (mg/g)	Sl. No.	RIL No.	PC (mg/g)	Sl. No.	RIL No.	PC (mg/g)
BPT 5204	C1	17.393	BPT 5204	C1	17.393	BPT5204	C1	17.393	BPT5204	C1	17.393
HPR-14	C2	23.966	HPR-14	C2	23.966	HPR-14	C2	23.966	HPR-14	C2	23.966
1	BH-RIL-00001	18.263	51	BH-RIL-00248	20.874	101	BH-RIL-00522	15.136	151	BH-RIL-00829	22.437
2	BH-RIL-00009	18.369	52	BH-RIL-00249	19.956	102	BH-RIL-00523	17.205	152	BH-RIL-00830	20.286
3	BH-RIL-00022	18.534	53	BH-RIL-00260	20.474	103	BH-RIL-00529	15.841	153	BH-RIL-00836	21.65
4	BH-RIL-00023	18.604	54	BH-RIL-00262	20.345	104	BH-RIL-00530	18.252	154	BH-RIL-00793	22.355
5	BH-RIL-00024	19.251	55	BH-RIL-00263	20.827	105	BH-RIL-00540	16.229	155	BH-RIL-00843	21.861
6	BH-RIL-00028	19.968	56	BH-RIL-00264	20.38	106	BH-RIL-00573	18.957	156	BH-RIL-00845	21.955
7	BH-RIL-00029	19.451	57	BH-RIL-00269	20.862	107	BH-RIL-00576	18.381	157	BH-RIL-00852	21.744
8	BH-RIL-00030	17.417	58	BH-RIL-00272	19.827	108	BH-RIL-00594	17.182	158	BH-RIL-00856	22.343
9	BH-RIL-00031	17.323	59	BH-RIL-00282	20.674	109	BH-RIL-00595	17.57	159	BH-RIL-00859	19.545
10	BH-RIL-00032	16.653	60	BH-RIL-00287	20.286	110	BH-RIL-00595	16.711	160	BH-RIL-00861	19.909
11	BH-RIL-00037	18.546	61	BH-RIL-00298	21.626	111	BH-RIL-00610	17.57	161	BH-RIL-00865	19.592
12	BH-RIL-00038	18.687	62	BH-RIL-00302	23.249	112	BH-RIL-00618	17.605	162	BH-RIL-00866	21.073
13	BH-RIL-00039	18.604	63	BH-RIL-00306	23.096	113	BH-RIL-00623	14.995	163	BH-RIL-00867	18.393
14	BH-RIL-00041	18.075	64	BH-RIL-00317	28.116	114	BH-RIL-00633	17.981	164	BH-RIL-00869	21.414
15	BH-RIL-00042	19.18	65	BH-RIL-00332	22.778	115	BH-RIL-00636	16.617	165	BH-RIL-00877	19.557
16	BH-RIL-00045	17.84	66	BH-RIL-00333	22.285	116	BH-RIL-00638	18.04	166	BH-RIL-00881	20.074
17	BH-RIL-00047	17.981	67	BH-RIL-00334	26.541	117	BH-RIL-00640	16.888	167	BH-RIL-00884	22.014
18	BH-RIL-00048	16.794	68	BH-RIL-00339	27.505	118	BH-RIL-00641	16.182	168	BH-RIL-00896	20.58
19	BH-RIL-00049	18.934	69	BH-RIL-00344	20.968	119	BH-RIL-00656	15.971	169	BH-RIL-00914	21.814
20	BH-RIL-00053	19.239	70	BH-RIL-00345	19.063	120	BH-RIL-00662	19.075	170	BH-RIL-00923	21.026
21	BH-RIL-00061	20.474	71	BH-RIL-00347	20.521	121	BH-RIL-00669	20.932	171	BH-RIL-00947	20.227
22	BH-RIL-00065	20.074	72	BH-RIL-00348	23.919	122	BH-RIL-00670	23.049	172	BH-RIL-00950	18.51
23	BH-RIL-00066	22.543	73	BH-RIL-00349	19.11	123	BH-RIL-00673	24.33	173	BH-RIL-00976	17.746
24	BH-RIL-00071	20.474	74	BH-RIL-00350	21.556	124	BH-RIL-00677	22.943	174	BH-RIL-01004	19.157
25	BH-RIL-00072	21.638	75	BH-RIL-00352	19.463	125	BH-RIL-00680	21.885	175	BH-RIL-01005	20.862
26	BH-RIL-00082	19.204	76	BH-RIL-00353	20.509	126	BH-RIL-00682	20.497	176	BH-RIL-01009	22.238
27	BH-RIL-00083	21.003	77	BH-RIL-00357	19.51	127	BH-RIL-00684	19.992	177	BH-RIL-01017	20.486
28	BH-RIL-00095	20.791	78	BH-RIL-00358	20.392	128	BH-RIL-00692	20.18	178	BH-RIL-01034	20.145

29	BH-RIL-00132	19.016	79	BH-RIL-00361	18.275	129	BH-RIL-00694	22.802	179	BH-RIL-01044	21.297
30	BH-RIL-00136	20.098	80	BH-RIL-00362	20.333	130	BH-RIL-00701	19.298	180	BH-RIL-01048	20.756
31	BH-RIL-00139	19.486	81	BH-RIL-00364	20.297	131	BH-RIL-00706	20.603	181	BH-RIL-00554	23.061
32	BH-RIL-00142	19.157	82	BH-RIL-00368	23.19	132	BH-RIL-00719	19.486	182	BH-RIL-01061	23.648
33	BH-RIL-00152	19.98	83	BH-RIL-00369	23.072	133	BH-RIL-00722	22.014	183	BH-RIL-01067	22.026
34	BH-RIL-00153	20.533	84	BH-RIL-00370	21.461	134	BH-RIL-00722	22.578	184	BH-RIL-01068	24.471
35	BH-RIL-00155	19.804	85	BH-RIL-00386	20.909	135	BH-RIL-00727	22.002	185	BH-RIL-01073	22.026
36	BH-RIL-00180	21.226	86	BH-RIL-00421	25.483	136	BH-RIL-00731	22.814	186	BH-RIL-01078	24.401
37	BH-RIL-00190	17.675	87	BH-RIL-00439	22.578	137	BH-RIL-00749	20.874	187	BH-RIL-01089	23.743
38	BH-RIL-00196	19.286	88	BH-RIL-00440	22.966	138	BH-RIL-00762	21.767	188	BH-RIL-01101	26.894
39	BH-RIL-00200	17.887	89	BH-RIL-00441	22.355	139	BH-RIL-00764	18.51	189	BH-RIL-01107	25.577
40	BH-RIL-00203	18.063	90	BH-RIL-00442	19.933	140	BH-RIL-00770	18.463	190	BH-RIL-01108	22.343
41	BH-RIL-00205	22.332	91	BH-RIL-00443	23.26	141	BH-RIL-00771	22.038	191	BH-RIL-01110	22.778
42	BH-RIL-00208	22.437	92	BH-RIL-00030	21.356	142	BH-RIL-00773	21.238	192	BH-RIL-01112	21.567
43	BH-RIL-00209	22.296	93	BH-RIL-00453	22.802	143	BH-RIL-00791	21.121	193	BH-RIL-01116	21.156
44	BH-RIL-00214	20.885	94	BH-RIL-00456	23.084	144	BH-RIL-00793	21.273	194	BH-RIL-01117	22.014
45	BH-RIL-00215	20.956	95	BH-RIL-00465	25.483	145	BH-RIL-00796	20.932	195	BH-RIL-01118	22.637
46	BH-RIL-00216	19.933	96	BH-RIL-00466	22.626	146	BH-RIL-00800	22.249	196	BH-RIL-01120	22.496
47	BH-RIL-00225	20.638	97	BH-RIL-00501	21.732	147	BH-RIL-00805	22.296	197	BH-RIL-00876	20.979
48	BH-RIL-00230	19.604	98	BH-RIL-00503	23.554	148	BH-RIL-00810	19.463	198	BH-RIL-01025	20.004
49	BH-RIL-00231	21.779	99	BH-RIL-00508	23.178	149	BH-RIL-00826	22.943	199	BH-RIL-01054	23.249
50	BH-RIL-00242	19.122	100	BH-RIL-00509	21.179	150	BH-RIL-00827	20.78	200	BH-RIL-01125	21.379

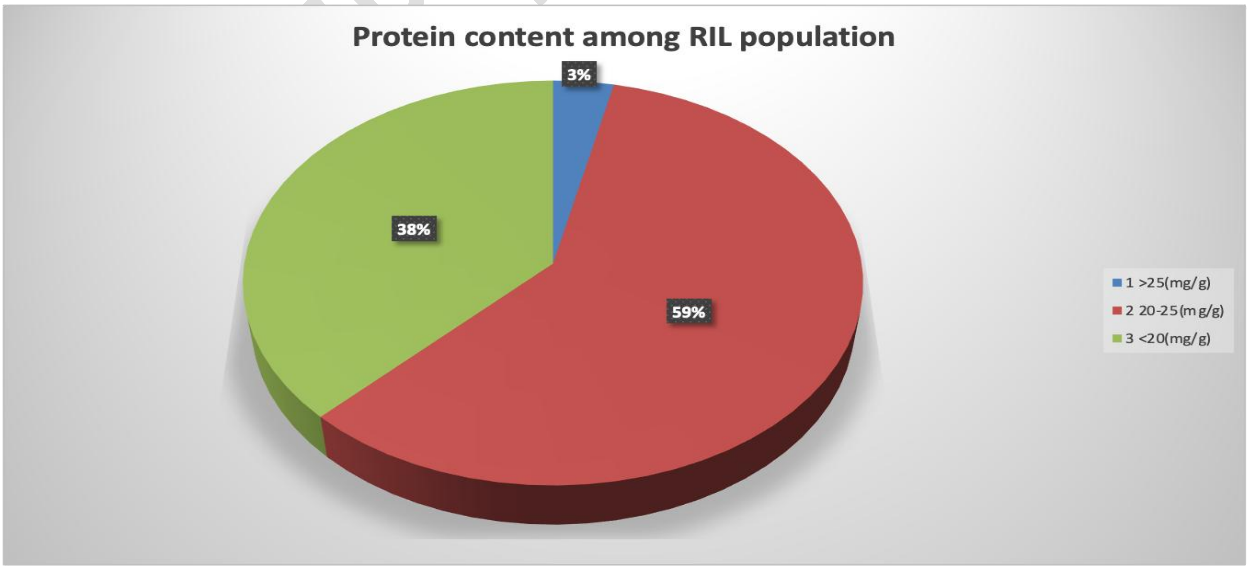

Fig.1: Pie chart representation of protein content among 200 recombinant inbred lines of rice

Table 2: Grouping of Recombinant inbred lines based on mean crude protein content

Crude protein content (mg/g)	Individuals	Number of Recombinant inbred lines	Per centage of individuals	*Classification
>25 (mg/g)	BH-RIL-00317, BH-RIL-00339, BH-RIL-01101, BH-RIL-00334, BH-RIL-01107, BH-RIL-00421, BH-RIL-00465	7	3 %	High
20 to 25(mg/g)	BH-RIL-01025, BH-RIL-00065, BH-RIL-00881, BH-RIL-00136, BH-RIL-01034, BH-RIL-00692, BH-RIL-00947, BH-RIL-00287, BH-RIL-00830, BH-RIL-00364, BH-RIL-00362, BH-RIL-00262, BH-RIL-00264, BH-RIL-00358, BH-RIL-00071, BH-RIL-00260, BH-RIL-00061, BH-RIL-01017, BH-RIL-00682, BH-RIL-00353, BH-RIL-00347, BH-RIL-00153, BH-RIL-00896, BH-RIL-00706, BH-RIL-00225, BH-RIL-00282, BH-RIL-01048, BH-RIL-00827, BH-RIL-00095, BH-RIL-00263, BH-RIL-01005, BH-RIL-00269, BH-RIL-00248, BH-RIL-00749, BH-RIL-00214, BH-RIL-00386, BH-RIL-00669, BH-RIL-00796, BH-RIL-00215, BH-RIL-00344, BH-RIL-00876, BH-RIL-00083, BH-RIL-00923, BH-RIL-00866, BH-RIL-00791, BH-RIL-01116, BH-RIL-00509, BH-RIL-00180,	119	59 %	Moderate

	BH-RIL-00773, BH-RIL-00793, BH-RIL-01044, BH-RIL-00030, BH-RIL-01125, BH-RIL-00869, BH-RIL-00370, BH-RIL-00350, BH-RIL-01112, BH-RIL-00298, BH-RIL-00072, BH-RIL-00836, BH-RIL-00501, BH-RIL-00852, BH-RIL-00762.BH-RIL-00231, BH-RIL-00914, BH-RIL-00843, BH-RIL-00680, BH-RIL-00845, BH-RIL-00727, BH-RIL-00884, BH-RIL-00722, BH-RIL-01117, BH-RIL-01073, BH-RIL-01067, BH-RIL-00771, BH-RIL-01009, BH-RIL-00800, BH-RIL-00333.BH-RIL-00209, BH-RIL-00805, BH-RIL-00205, BH-RIL-00856, BH-RIL-01108, BH-RIL-00441, BH-RIL-00793, BH-RIL-00208, BH-RIL-00829, BH-RIL-01120, BH-RIL-00066, BH-RIL-00722, BH-RIL-00439, BH-RIL-00466, BH-RIL-01118, BH-RIL-00332, BH-RIL-01110, BH-RIL-00694, BH-RIL-00453, BH-RIL-00731, BH-RIL-00677, BH-RIL-00826, BH-RIL-00440, BH-RIL-00670, BH-RIL-00554, BH-RIL-00369, BH-RIL-00456, BH-RIL-00306, BH-RIL-00508, BH-RIL-00368, BH-RIL-00302, BH-RIL-01054, BH-RIL-00443, BH-RIL-00503, BH-RIL-01061, BH-RIL-01089, BH-RIL-00348, **HPR-14**, BH-RIL-00673, BH-RIL-01078, BH-RIL-01068			
<20 (mg/g)	BH-RIL-00623, BH-RIL-00522, BH-RIL-00529, BH-RIL-00656,	76	38 %	Low

	BH-RIL-00641, BH-RIL-00540, BH-RIL-00636, BH-RIL-00032, BH-RIL-00595, BH-RIL-00048, BH-RIL-00640, BH-RIL-00594, BH-RIL-00523, BH-RIL-00031, **BPT-5204,** BH-RIL-00030, BH-RIL-00595, BH-RIL-00610, BH-RIL-00618, BH-RIL-00190, BH-RIL-00976, BH-RIL-00045, BH-RIL-00200, BH-RIL-00633, BH-RIL-00047, BH-RIL-00638, BH-RIL-00203, BH-RIL-00041, BH-RIL-00530, BH-RIL-00001, BH-RIL-00361, BH-RIL-00009, BH-RIL-00576, BH-RIL-00867, BH-RIL-00770, BH-RIL-00764, BH-RIL-00950, BH-RIL-00022, BH-RIL-00037, BH-RIL-00039, BH-RIL-00023, BH-RIL-00038, BH-RIL-00049, BH-RIL-00573, BH-RIL-00132, BH-RIL-00345, BH-RIL-00662, BH-RIL-00349, BH-RIL-00242, BH-RIL-01004, BH-RIL-00142, BH-RIL-00042, BH-RIL-00082, BH-RIL-00053, BH-RIL-00024, BH-RIL-00196, BH-RIL-00701, BH-RIL-00029, BH-RIL-00352, BH-RIL-00810, BH-RIL-00139, BH-RIL-00719, BH-RIL-00357, BH-RIL-00859, BH-RIL-00877, BH-RIL-00865, BH-RIL-00230, BH-RIL-00155, BH-RIL-00272, BH-RIL-00861, BH-RIL-00216, BH-RIL-00442, BH-RIL-00249, BH-RIL-00028, BH-RIL-00152			