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A multiplexed allele-specific assay for high-throughput genotyping of 22 perfect markers of important agronomic and quality traits in soybean for assisting germplasm characterization and molecular breeding

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The allele specific functional DNA markers are the answer to precisely select multiple traits from multiple parents without phenotyping. A high throughput and accurate DNA marker genotyping method is desirable for genotyping several number of allele specific markers in large breeding populations. Therefore a multiplexing amenable multi-trait allele specific SNP/Indel panel was developed for high throughput genotyping of soybean germplasm accessions and breeding populations. A total of 14 genes were selected for 8 different agronomic and quality traits namely; flowering, pod shattering, hard-seededness, growth habit, long juvenile, phosphorous efficiency, salt tolerance, oleic acid content and fragrance. A total of 28 alleles known to be associated with phenotypic variance among 14 genes were selected for SNP/Indel detection. Out of 28 allele sequences, primers for 23 alleles were incorporated in single assay for genotyping using matrix-assisted laser desorption ionization-time of flight mass spectrometry. The 23 allele multiplex assay was used for genotyping of 192 samples comprising 164 germplasm accessions selected from core set, one accession of *Glycine soja*, eight parental genotypes of a multi-parent advance generation inter-cross (MAGIC) population, two vegetable type soybean genotypes, one high oleic genotype, and four other trait specific genotypes. The results indicated an overall success rate of 98% for 22 alleles while one marker failed to amplify. Out of 22 primers, 10 primers detected rare and minor alleles ranging from 1 to 62, among 180 soybean genotypes. Among the parents of MAGIC population, polymorphism was detected for five alleles associated with five different traits. Therefore, this high-throughput multiplex assay can be used for combining useful alleles of five different traits from eight genotypes. Among parents of two RIL populations, six alleles for four different traits were found polymorphic which can be used for selection of early maturity, shattering resistance, growth habit and salt tolerance traits.