Groundnut (Arachis hypogaea L.) is one of the principal economic crops of the world. Seed is the main edible part of the plant and rich source of oil, protein, carbohydrates, minerals and vitamins. It was introduced into India during the first half of the sixteenth century from one of the Pacific islands of China. The major groundnut-producing countries of the world are India, China, Nigeria, Senegal, Sudan, Burma and USA. It is a self-pollinated, annual, herbaceous, allotetraploid legume (2n=4x=40) with a genome size of 2891 Mbp and belongs to the subfamily papilionacea of the family Fabaceae.

Flowering is one of the most important agronomic traits manipulating crop yield. Thus, transcriptome, metabolomics and proteomics approach was applied to understand gene expression dynamics, changes in metabolites and proteins during flower development stages in groundnut genotype GJG-22; namely stage 1: bud, first tetra foliate leaf [Upper Leaf (UL1)], last tetra foliate leaf [Lower Leaf (LL1)] and main internode bearing primary branch [Stem (S1)]; stage 2: flower, upper leaf 2 (UL2), lower leaf 2 (LL2) and stem 2 (S2); stage 3: peg, upper leaf 3 (UL3), lower leaf 3 (LL3) and stem 3 (S3).

To study the transcriptome of groundnut, after completions of sequencing run the total raw sequence generated by Ion S5 sequencer were assessed through FASTQC quality control tool in which all 12 (twelve) samples having good quality sequence were selected for further analysis. Trimming of raw reads yielded a total of 16388597, 8857434 and 7619308 reads in bud, flower and peg, respectively. 9399136, 21154669 and 6439197 reads were yielded in UL1, UL2 and UL3, respectively. 9449809, 4096971 and 17852654 reads were yielded in LL1, LL2 and LL3, respectively. 2698800, 3187629 and 17449479 reads were yielded in S1, S2 and S3, respectively.

In differential expression analysis, combined cluster of 1, 2, 3, 4 and 5 having total number of 32833 gene cluster in bud, flower and peg; 49477 gene cluster in S1, S2 and S3; 49474 gene cluster in UL1, UL2 and UL3; 49477 gene cluster in LL1, LL2 and LL3; were up/down regulated listed only top 5 highly up/down regulated gene cluster.

Expression of auxin response factor 2A protein (ARF2-A_2) was up regulated in bud and minor change in expression showed in flower which represent floral organ abscission and positive regulation of flower development, while down regulated in peg, like BNH protein...
pectinesterase – like pollen specific protein (AT3G13400) was highly up regulated in flower which present at mature pollen stage (anthesis) but down regulated in bud and peg. Dehydrin protein (COR47) was showed highly up regulated in peg which represent defense response to fungus; down regulated in bud and flower. Xyloglucan endotransglycosylase 6, GDSL esterase/lipase, syntaxin/t-SNARE family protein, phosphoglyceride transfer family protein, cupredoxin superfamily similar protein 12 , Aquaporin PIP1-5, UDP-glucose 4-epimerase 1, 1-aminocyclopropane-1-carboxylate oxidase 3 and ubiquitin-60S ribosomal protein were up-regulated in S3 and down regulated in S1 and S2. Ribulose bisphosphate carboxylase, actin A3 protein, photosystem II light harvesting complex protein and chlorophyll a-b binding protein were mostly up-regulated in UL2 and down regulated in UL1 and UL3. Polyubiquitin 14, mediator of RNA polymerase II transcription subunit 37c, heat shock protein HSP 90-beta, molecular chaperone Hsp90-1, polyubiquitin 11 and ubiquitin 13 were up-regulated in UL3 while down regulated in UL1 and UL2. Phytophthora-inhibited protease, ubiquitin-60S ribosomal protein, H'-ATPase 6, polyubiquitin 14, 1-aminocyclopropane-1-carboxylate oxidase, ankyrin repeat family protein, glyceraldehyde-3-phosphate dehydrogenase, xyloglucan endotransglucosylase/hydrolase, L-ascorbate oxidase pectinesterase-like protein, reticulon-like protein, ADP-ribosylation factor and metallothionein-like protein like all were up-regulated in LL3 sample and down-regulated in LL1 and LL2. Furthermore, the selected genes were validated through qRT PCR analysis.

Based on the gene ontology (GO) analysis, total GOs covers three domains: 50-biological process (BP), 40-molecular functional (MF) and 35-cellular component (CC) in all the tissues. For enzyme code distribution, maximum sequence were found for Hydrolases.

In KEGG pathways, the pathways with unique sequences were observed. These results included pathways related to bud, flower and peg development like, copper ion binding, oxidoreductase activity, oxidizing metal ions, auxin-activated signaling pathway, transcription factor activity, sequence-specific DNA binding and many other molecular pathways.

Many EST-SSR were identified which serves as a resource of high quality transcripts for gene discovery and development of functional markers associated with different stages of flower development. In the present study, sequence collection represents a major transcriptomics resource for groundnut and the large number of genetic markers predicted should contribute to future research.

In metabolomic profiling by GC-MS, generalize metabolome regulations like sugars, alcohols, glucosides, esters, alkanes and sugar alcohols, organic acids, and fatty acids were analyzed. Some other metabolites like amino acids and polyamines also analyzed. malic acid, succinic acid and fumaric acid, significantly increased in bud to flower to peg, which indicated that respiration at peg stage was higher, metabolic activity increase, and growth and development accelerated. Organic acids and fatty acids were the metabolites that exhibited a high degree of variance during stage 3, increases in levels of benzoic acid, tartaric acid, butanoic acid, malic acid, propanoic acid, gluconic acid, nonanoic acid, heptadecanoic acid, octadecanoic acid, oleic acid, myristic acid and 11-Eicosenoic acid. Identified homocysteine in stage 3, the immediate precursor of methionine, is a key reaction in biology. L-ascorbic acid and aspartic acid showed increased levels in UL3. Some non-targeted metabolites like alkaloid, flavonoids, triterpenoids and fatty acyl that may have different metabolic and signaling functions. Up-regulated soyasaponin observed in peg, where down regulated in bud. Interestingly, up regulated of several metabolites was also observed in stage 3 were identified using LC-MS Q-TOF.

The protein profiling were also compared at different flower development stages of groundnut. Comparative analysis of spots revealed the few proteins were up-regulated in stage 3. Protein revealed that total 9193 protein spots with the pH range 4 - 7 and 23-312 kDa range were recorded. Out of 9193 spots, maximum 3413 spots were found in pH range 6 - 7 in groundnut samples.

The present combinatorial study of transcriptomics, metabolomics and proteomics provided the variable molecular insights in the mechanism of flower development stages in groundnut.