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Effect of heat use efficiency on bioproduction of drought resistance eliciting phytochemicals in groundnut (*Arachishypogaea* L.) genotypeGKVK-5

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ABSTRACT

Thermal utilization factors are crucial throughout the crop period, from seedling emergence to maturity, significantly affecting crop yield. Groundnut (ArachishypogaeaL.) shows drought tolerance through secondary metabolites, helping maintain biomass during arid conditions. This study compared heat use efficiency with the HR-LCMS profiling of phytochemicals in groundnut genotype GKVK-5. A field study was conductedon sandy loam soils at AHRS, Bavikere, UAHS, Shivamogga, Karnataka, India, under rain-fed circumstances during the (Kharif)season. Results showed GKVK-5 had a total dry matter of over 18.75g per plant, with an increased HUE of 14.56g ºC day^{-1} at 90 days after sowing. Higher HTUE (2.258 q ha^{-1} HTU^{-1}) resulted in more pod output (16.73 q ha^{-1}) and haulm yield (26.99 q ha^{-1}) compared to TMV 2 (10.48 q ha^{-1} and 15.07 q ha^{-1}). The increase in HUE demonstrates GKVK-5's tolerance to drought stress. During a qualitative phytochemical screening, GKVK-5's leaf methanol extract tested positive for alkaloids, flavonoids, and phenolics. The quantitative estimation yielded values of 32.44µg/mg for total alkaloids, 38.61µg/mg for flavonoids, and 47.32µg/mg for phenolics.HR-LCMS profiling identified drought resistance-eliciting phytochemicals, such as 2-Amino-3-methylhexanoic acid, 6-Deoxyfagomine Maltoxazine, and 2-Furanyl pyrrolidine, in cation mode. Antioxidant flavonoids Astragalin 7-rhamnoside, Naringenin 7-O-glucoside, and PhenolicsRutin, Gallic acid, and quinic acid were detected in anion mode using high DB hits, retention duration, and MS spectra. These oxidative stress molecules led to enhanced heat use efficiency and drought stress adaptation in genotype GKVK-5.

Keywords: *Groundnut, HUE, HR-LCMS profiling, Drought stress adaptation Short title:Heat use efficiency and oxidative stress compounds of groundnut.*

INTRODUCTION

Heat stress caused by increased temperature is a major problem in world agriculture. Plants experience a range of morpho-anatomical, physiological, and biochemical changes in response to brief or persistently high temperatures. These changes can have a significant effect on the growth of plants. and development and potentially result in a sharp decline in economic yield. Genetic factors, crop type, and the crop's capacity for the biosynthesis of secondary metabolites during dry seasons and drought all affect how well heat is utilized in terms of dry matter accretion. The world's most significant oilseed and cash crop, groundnut (*Arachishypogaea L.)*, is grown in semi-arid regions. However, fluctuations in the agroclimatic conditions and the chemical composition of biological components directly impact the crop's growth and output.In Central Punjab, India, the studies of [1] have documented the impact of sowing dates on the groundnut cultivar's thermal use and heat use efficiency.

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The thermal requirements and heat consumption efficiency under various sowing circumstances and row orientations were also reported in pea cultivars [2].. A delay in crop sowing significantly reduced the length of phenological stages and the accumulation of agro-climatic indices (GDD, PTU, and HTU).The researchers noticed that the plants sustained in drought due to biosynthesis of the key secondary metabolite molecules and plant metabolic responses to drought stress have been comprehensively profiled by metabolic profile analysis [3]. Thus, utilizing the environmental parameters of growing degree days (GDD), heliothermal units (HTU), and photothermal units (PTU), an attempt was made to assess the growth and yield of groundnut genotypes GKVK-5 and TMV-2 in the current study.
Many secondary metabolites are essential for plants to adapt and increase their chances of survival during drought and other environmental stresses.Hence analysis of metabolic profiles of the crop plant and their role in drought tolerance has sought attention towards the improvement of crop plants.Hence, in the present study, HR-LCMS analysis was carried out to identify phytochemicals of *A.hypogaea*thatplay a significant role in drought tolerance and oxidative stress.

MATERIAL AND METHODS

Heat use efficiency of groundnut genotypes:

A field study was carried out under rainfed conditions during the *Kharif*seasonon sandy loam soils of AHRS, Bavikere, UAHS, Shivamogga (13º 42' latitude and 75 º 51' longitude and 695 m above MSL). The experiment was laid out in a whole block design that is randomized with a factorial concept[4].

Testing was done on the groundnut genotypes, GKVK-5 and TMV-2, as well as the four sowing windows, which are the second and first weeks of June, July, and August.For each genotype experiments were conducted with three replications. Seeds were sown at a depth of 5 cm with 30 x 15 cm spacing. The recommended dose of fertilizers (25: 50: 25 kg N: P_2O_5: K_2O) was applied as a basal dose (50 % N). The remaining 50 % of N was applied during the time of earthing up (30 DAS). The weather parameters were obtained from AHRS, Bavikere. The HUE (Heat use efficiency) and HTU (Helio-thermal units) were calculated from GDD obtained using the weather parameters.

HTU is the product of accumulated heat units and corresponding actual sunshine hours, which was calculated using the formula,

HTU = $\sum$GDD x Actual sunshine hours (0 to 90 DAS)

HTUE is the ratio of yield and HTU,

$$\text{HTUE} = \frac{\text{Yield}}{\text{HTU}}$$

HUE is the ratio of total dry matter to the accumulated GDD,

$$\text{HUE} = \frac{\text{Total dry matter}}{\sum \text{GDD}}$$

Based on the result of HUE and HTU experiments the drought-resistant genotype was subjected to qualitative and quantitative phytochemical screening.

Preparation of Extract

The fresh and healthy leaves of the ground nut genotypes GKVK-5 were collected from the experimental plots of AHRS, Bavikere, UAHS, and Shivamogga. About 30 g of powdered leaves of each genotype were subjected to sequential Soxhlet extraction using the solvents, petroleum ether, chloroform, and methanol. The extracts were sieved through Whatman paper no.1 and then concentrated using a rotary flash evaporator.

Qualitative phytochemical screening

The dried residue of petroleum ether, ethyl acetate, and methanol extracts were resuspended in Mili-Q water and qualitatively examined for various secondary metabolites viz., alkaloids, flavonoids, terpenoids, glycosides, saponins, phenols using standard tests [5].

Quantitative estimation of phytochemicals

Estimation of total flavonoid content in sequential extracts of leaves of ground nut was determined [6]. The amounts of total phenolic contents of extracts were determined by the spectrophotometric method[7]. Estimation of total alkaloids was performed by gravimetricmethod [8] and total terpenoids in the extracts was also determined by spectrophotometric method [9].

HR-LCMS analysis of leaves methanol extract

A High-resolution liquid chromatograph mass spectrometer (HR-LCMS) G6550A (Agilent technologies) instrument was used to examine the bioactive components of groundnut genotypes of leaves methanol extract. The method used for chromatography was 30 mins ± ESI 10032014_MSMS.m. The temperature of the gas utilized for analysis was 250 °C. Identification was done using the protonated compound's theoretical mass. The compounds expressed with more than 10 Hits were considered for comparative evaluation. The lead compounds expressed repeatedly were used for spectral studies and characterization. HR-LC-MS analysis was performed at the Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology, Mumbai, India. The compounds were identified through a comparison of their mass and retention time (RT) with the stored Metlin Library maintained at IIT Bombay. The ESI's positive and negative ionization modes were used for the MS analysis. Gas temperature of 250 °C, drying gas flow of 13 L/minute, nebulizer pressure of 35 psi, and capillary voltage of 3,500 V were the parameters of the MS source that were employed. Acquisition of Q-TOF data and mass spectrometric analysis were done using Agilent Mass Hunter software.

RESULTS AND DISCUSSION

Influence of different sowing windows on GDD of groundnut crop

Weather data obtained from the Agricultural and Horticultural Research Station, Bavikere, Karnataka, India (Fig. S1) showed minimal variation in actual maximum and minimum temperatures and relative humidity from normal values, while rainfall and sunshine hours varied significantly. The computed weather parameter, GDD, was derived from these temperatures and varied accordingly (Table 1). Groundnut sown in the second fortnight of June recorded higher GDD (1721.95 ºC day-1) at harvest, decreasing with delayed sowing.

Groundnut dry matter depends on environmental factors like solar radiation, temperature, relative humidity, and moisture availability. The early sown crop (second fortnight of June) received more sunshine, rainfall, and longer day-length conditions, allowing it to intercept more radiation, utilize more moisture, and produce more photosynthates. Similar results, was also noticed by the previous investigator [10] where delayed sowing extended the time required for 50% of the population to initiate flowering.

Table 1. Influence of different sowing windows on Growing Degree Days of groundnut genotypes

Sowing windows (S)	Growing degree days (°C day^{-1})			
	0-30 DAS	0-60 DAS	0-90 DAS	0-At harvest
S_1: II Fortnight of June	433.48	861.66	1296.70	1721.95
S_2: I Fortnight of July	432.38	858.30	1290.24	1706.96
S_3: II Fortnight of July	428.18	863.22	1288.47	1696.07
S_4: I Fortnight of August	425.92	857.86	1274.58	1679.80

DAS: Days After Sowing

An increase in the total dry matter accumulation significantly increased the heat use efficiency (HUE) between the genotypes at 60 DAS and 90 DAS in GKVK-5 (Table 2). Similarly, an increase in the efficiency adds up to the increased photosynthates accumulation and further increases the pod yield. Thus, the genotype GKVK-5 produced a higher pod yield (16.73q ha-1). The decrease in the HUE in TMV-2 might be due to the decrease in total dry matter content which further decreased the pod yield. The increase in the total dry matter content increased the haulm yield in GKVK-5 (26.99 q ha-1), while it decreased in TMV-2 (15.07 q ha-1). This might be due to the stay-green nature of the varieties GKVK-5[11 and 12].. The increase in yield and total dry matter was noticed in the genotype GKVK-5 due to increased Helio-thermal use efficiency (HTUE) indicating the drought-resistant characteristics GKVK-5 to genotypes TMV-2, when sown during the second fortnight of June compared to delay sowing.

The crop sown during the second fortnight of June had taken more days for initiation of flower in the 50 percent of the population,where there was initially a better accumulation of the dry matter and could thus produce a higher pod yield of 15.2 q ha-1 (Table 2). An increase in the HUE was observed during 60 DAS and 90 DAS due to a drastic increase in the total dry matter content and GDD during the period. An increase in total dry matter provides a pre-requisite to increase the yield in any crop, as it indicates the maximum translocation of photosynthates to the sink. Based on the results of the HTUE experiment, the genotype GKVK-5 has been subjected to phytochemical investigation for the identification of metabolites that act as elicitors for acquiring drought-resistant characteristics.

Qualitative and quantitative phytochemical analysis

Preliminary qualitative phytochemical screening of the crude extracts (LPE-Leaves Petether Extract, LEE-Leaves Ethyl AcetateExtract, LME-Leaves Methanol Extract,) showed that alkaloids, terpenoids, and saponins were present in LPE. The LEE showed a positive test for alkaloids, Flavonoids, phenolics, terpenoids, and glycosides, whereas, tannins and saponins are absent. In LME except terpenoids all the phytochemical groups were present. In the quantitative determination of compounds,concentration of total alkaloids in LPE, LEE, and LME was estimated as 12.32, 36.24, and 32.44 μg/mg respectively. In LPE flavonoids and phenolics were absent. The concentration of total flavonoids in LEE and LME was38.61 and 28.56 μg/mg respectively similarly the total phenolics were estimated as 24.02 μg/mg in LEE and 47.32 μg/mg in LME. In the quantitative estimation of compounds, the LME showed higher concentrations of total alkaloids, and phenolic compounds therefore, the LME was subjected to HRLCMS analysis for the identification of compounds.

HR-LCMS analysis of leaves methanol extract of groundnut genotype GKVK-5

Based on the results of the HTUE experiment, the genotype GKVK-5 has been subjected to phytochemical investigation for the identification of metabolites that act as elicitors for acquiring drought-resistant characteristics. Secondary metabolites play an important role in the drought stress tolerance of crop plants.According to the previous investigator [12], under field conditions, plants are subjected to a variety of abiotic stressors. Drought stress, in particular, has an adverse effect on crop output and poses a threat to world food security. Drought stress activates downstream processes, including signaling pathways and phytohormone homeostasis.This in turn triggers the synthesis of many protective secondary metabolites. Multiple stress tolerance, including resistance to biotic and abiotic stressors, is provided by these secondary metabolites.

Table 2. Effect of sowing windows on total dry matter, HUE, HTUE, and yield of groundnut genotypes

Treatment	Total dry matter (g plant^{-1})				HUE x 10^{-3} (g ºC day^{-1})				HTUE x 10^{-3}				Yield	
	30 DAS	60 DAS	90 DAS	At harvest	0-30 DAS	0-60 DAS	0-90 DAS	0-At harvest	0-30 DAS	0-60 DAS	0-90 DAS	0-At harvest	Pod yield (q ha^{-1})	Haulm yield (q ha^{-1})
Genotypes (G)														
G_1: GKVK-5	1.99	12.99	18.75	20.84	4.62	15.10	14.56	12.24	7.567	3.434	203.25	1.669	16.73	26.99
G_4: TMV-2	1.90	9.91	14.57	15.83	4.42	11.52	11.32	9.30	4.737	2.152	127.26	1.045	10.48	15.07
S.E m±	0.04	0.13	0.13	0.27	0.09	0.15	0.10	0.16	0.093	0.041	0.0074	0.020	0.20	0.40
C.D. (p=0.05)	NS	0.37	0.36	0.78	NS	0.43	0.28	0.45	0.270	0.120	0.0025	0.058	0.59	1.16
Sowing windows (S)														
S_1: II fortnight of June	2.02	12.73	18.38	20.21	4.65	14.77	14.18	11.74	8.937	3.447	202.92	1.574	15.20	24.15
S_4: I fortnight of August	1.86	10.29	15.11	16.57	4.37	11.99	11.86	9.86	3.849	2.258	128.42	1.125	11.57	20.16
S.Em±	0.04	0.13	0.13	0.27	0.09	0.15	0.10	0.16	0.093	0.041	0.0074	0.020	0.20	0.40
C.D. (p=0.05)	NS	0.37	0.36	0.78	NS	0.43	0.28	0.45	0.270	0.120	0.0025	0.058	0.59	1.16
Interaction (G × S)														
S.Em±	0.08	0.25	0.25	0.54	0.19	0.30	0.20	0.31	0.187	0.083	0.0148	0.040	0.41	0.81
C.D. (p=0.05)	NS	0.74	0.73	NS	NS	0.85	0.57	NS	0.540	0.239	0.0049	0.117	1.18	2.33

DAS: Days after sowing NS: Non-significant

The HRLCMS of leaves methanol extract of groundnut genotype GKVK-5 showed expression of drought resistance eliciting compounds in both cation and anion mode spectra as shown in (Table 3).The greatest DB hits, longer retention times, and m/z values are expressed for these compounds. (Fig 1).Displays the mass, structure, and MS spectra of the cation compounds.

6-Deoxyfagomine is a polyhydroxylatedpiperidine alkaloid that occurs naturally and is an iminosugar, or pseudo-sugar with an N atom, with intriguing biological characteristics.

The compound isolated from Morusbompycis, exhibits strong antihyperglycemic impact on streptozotocin-induced diabetic mice and also increased the glucose-induced insulin secretion [13]. Maltoxazine, sometimes referred to as daechualkaloid A, is a member of the pyrrolidine class of chemical compounds. Among the HRLCMS-based identified compounds, L-2-Amino-3-methylenehexanoic acid (AMHA) was expressed with repeated folds (Table 3) indicating its synthesis in the groundnut genotype, during drought stress conditions. AMHA, an endogenous (2S, 3S)-α-amino acid found in nature (Fig. 1B), displays strong anti-extreme temperature stress activity in a variety of plant species. It also induces plant resistance, making it extremely effective against bacterial, viral, and fungal infections.

Table 3. Highest DB Hits Cation and Anion compounds resulted from the HR-LCMS of Ground nut leaves methanol extract.

Compound Name	Formula	RT	m/z	Mass	DB Diff (ppm)	Hits (DB)
Cation compounds						
6-Deoxyfagomine	$C_6H_{13}NO_2$	1.457	154.084	131.0948	-1.28	10
L-2-Amino-3-methylene-hexanoicacid	$C_7H_{13}NO_2$	2.99	166.084	143.0949	-2.04	10
L-2-Amino-3-methylene - hexanoicacid	$C_7H_{13}NO_2$	3.355	166.084	143.0949	-1.94	10
Maltoxazine	$C_{10}H_{13}NO_2$	6.739	202.085	179.0957	-6.16	10
(2-Furanyl)pyrrolidine	$C_8H_{11}NO$	6.739	160.079	137.0847	-4.62	10
Anion compounds						
Quinic acid	$C_7H_{12}O_6$	1.08	191.055	192.062	3.84	10
Naringenin7-O-glucoside	$C_{21}H_{22}O_{10}$	5.36	433.133	434.120	1.91	10
Astragalin7-rhamnoside	$C_{27}H_{30}O_{15}$	6.16	593.151	594.158	0.49	10
Rutin	$C_{27}H_{30}O_{16}$	7.32	608.145	610.152	0.93	10
Chicoric acid	$C_{22}H_{18}O_{12}$	7.54	473.072	474.079	0.31	10
Chicoric acid	$C_{22}H_{18}O_{12}$	7.85	473.072	474.079	0.81	10
AzukisaponinIV	$C_{48}H_{76}O_{20}$	10.64	971.485	972.492	0.71	10
SoyasaponinA3	$C_{48}H_{78}O_{19}$	12.09	957.506	958.513	0.14	10
PisumsaponinII	$C_{48}H_{76}O_{18}$	13.00	939.496	940.503	-0.18	10
HodulosideVIII	$C_{46}H_{76}O_{18}$	13.41	961.494	916.493	10.08	10

AMHA, an endogenous (2S, 3S)-α-amino acid found in nature (Fig. 1B), displays strong anti-extreme temperature stress activity in a variety of plant species. Additionally, it induces plant resistance, making it extremely effective against bacterial, viral, and fungal infections. According to [14], AMHA pretreatment provided excellent protection against powdery mildew in wheat, pseudomonas syringae DC3000 in Arabidopsis, and tomato spotted wilt virus in tobacco. With its exceptional potential, AMHA could develop into a special natural elicitor that shields plants from both biotic and abiotic stressors. 2-Amino-3-Methylhexanoic acid is a naturally occurring plant inducer that shields physiological function from the damaging effects of high temperatures on *Camellia sinensis* [15]. As a result, there is a lot of potential for AMHA to be developed as a naturally occurring commercial plant drought-resistance inducer. Plant cells biosynthesize a significant amount of secondary metabolites from primary metabolites.Plants produce secondary metabolites as a means of adaptation to deal with a variety of biotic and abiotic stressors [16].

In the present study also anion mode of HRLCMS resulted the presence offlavonoid glycosides- Astragalin 7-rhamnoside (Fig.2B), and phenolic compounds,rutin (Fig 2C) and chicoric acid (Fig 2D). These compounds were expressed with the highest DB hits, more retention time, and m/z value (Table 3). A marked accumulation of phenolic compounds chicoric acid chlorogenic acid, and flavonoid compounds. Quercetin-3-O-glucoside and luteolin-7-O-glucoside were also noticdin Lettuce during environmental stresses [17].In the previous study we also noticed that groundnut genotype GKVK-5 showed increased thermal utilization efficiency when it was cultivated in the delayed monsoon season. Drought stress is caused by high transpiration rates combined with low water availability in plants, which lowers their turgor pressure and water potential [18]. Consequently, alteration occurs in the bioproduction of secondary metabolites.The cation mode accumulation of flavonoid compounds, such as astragalin 7-rhamnoside and phenolic compounds, and non-protein amino acid, 2-Amino-3-methylhexanoic acid. In the groundnut genotype GKVK-5, chicoric acid and saponin compounds—Azukisaponin IV, Soyasaponin A3, and Pisumsaponin II—in an anion mode contributed to the development of drought resistance. Strong antioxidant Naringenin-7-O-glucoside down-regulated 3T3-L1 cell diameter and up-regulated intracellular lipid accumulation and adiponectin release. A flavonoid-7-o-glycoside called astragalin 7-rhamnoside may function as a signaling or defense molecule in a variety of crop species.In many crop species, it plays a major role in acquiring resistance against abiotic and biotic stress [11].

The study concludes that the groundnut genotype GKVK-5 showed increased heat use efficiency and drought stress adaptable characteristics during the second fortnight of June due toits enhanced secondary metabolites production.

Conflict of interest

The authors declare that there are no conflicts of interest associated with this publication.

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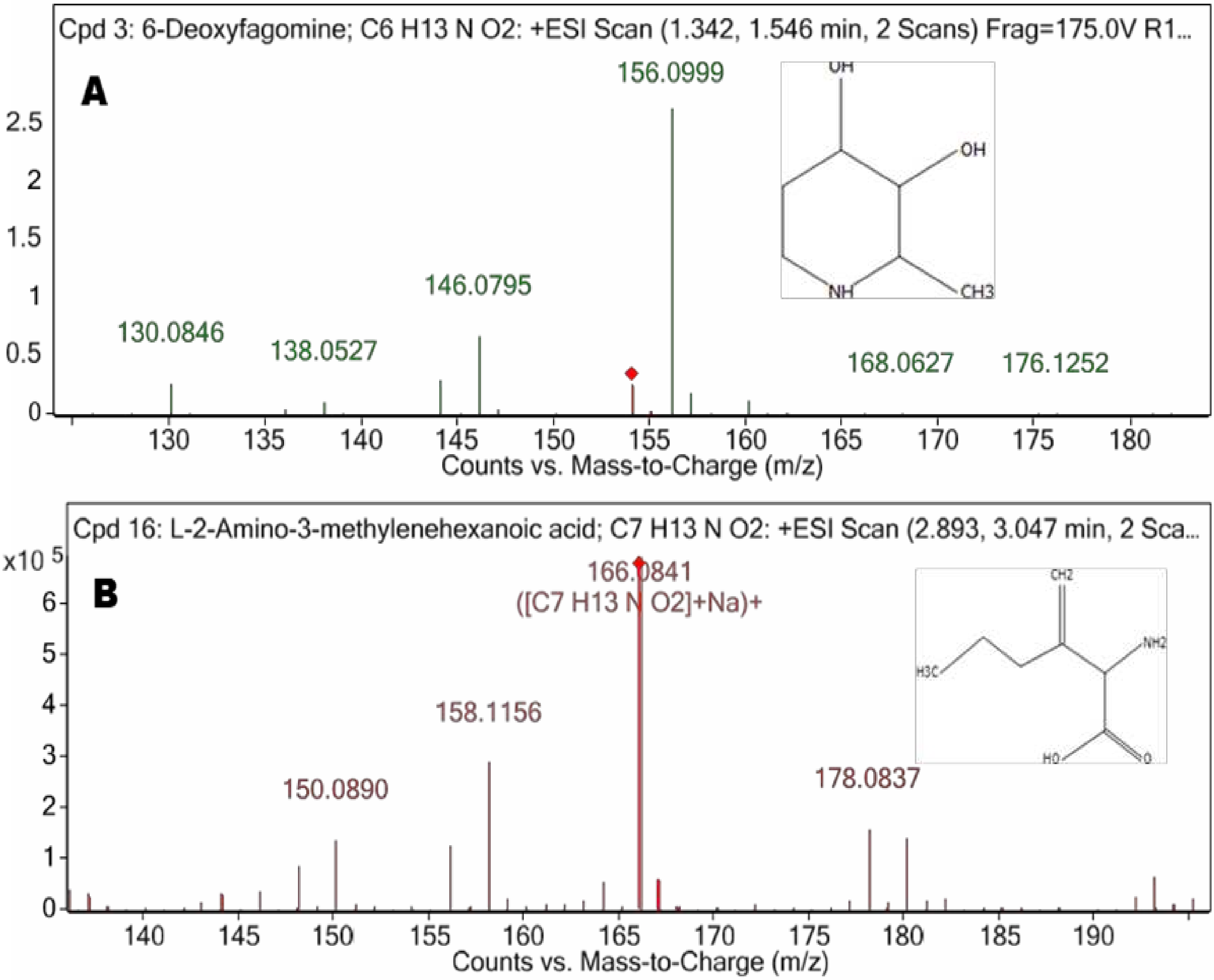
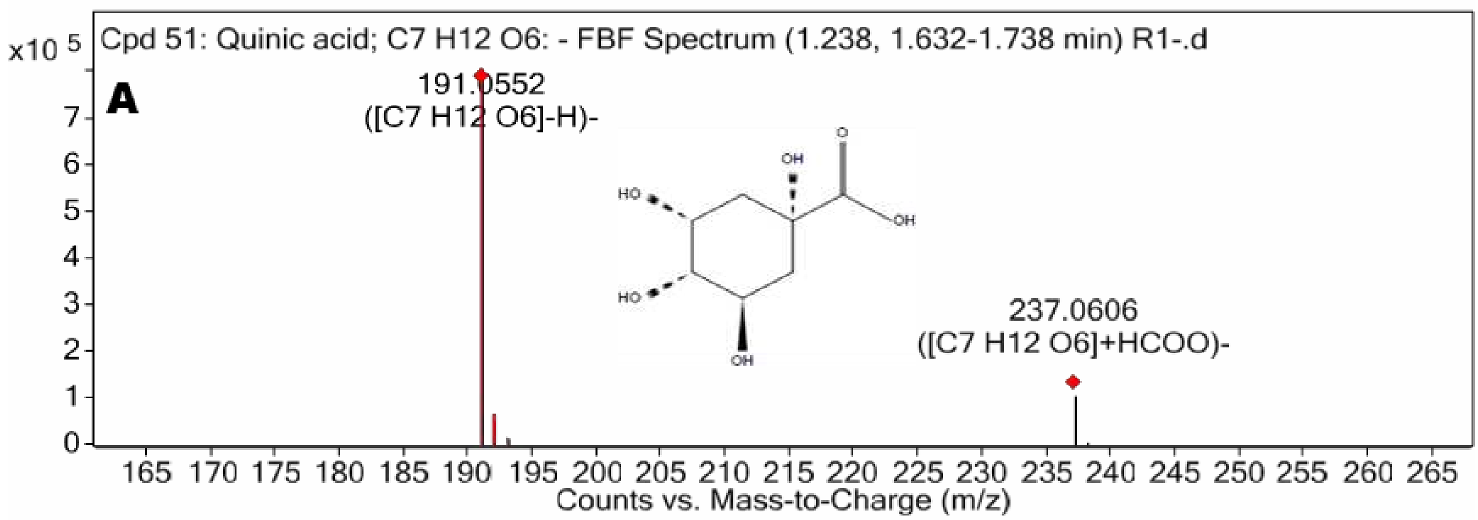
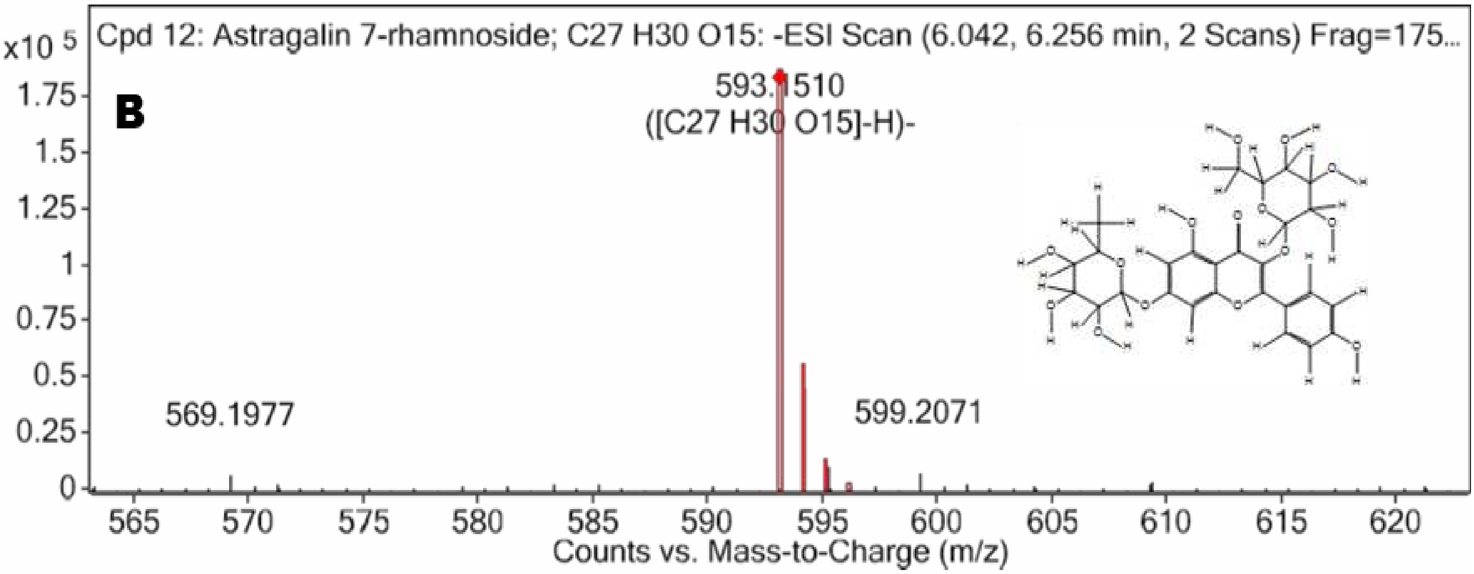
***Fig.1**(A,B) The spectra highlight the identification of compounds such as 6-Deoxyfagomine, and L-2-Amino-3-methylenehexanoic acidfrom the HR-LCMS analysisof groundnut genotype GKVK-5.*

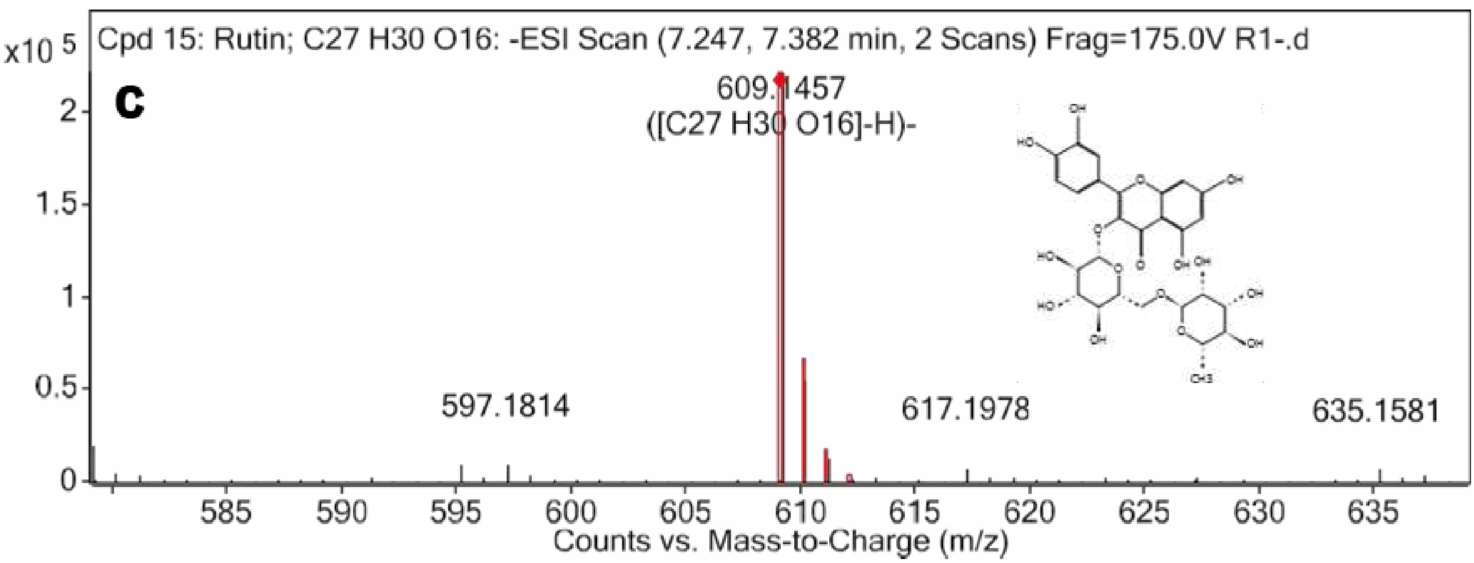


***2(A,B,C,D)** The spectrashow the identification of compounds such as Quinic acid,Astragalin 7-rhamnoside,Rutin and chicoricacid respectively from the HR-LCMS analysis of groundnut genotype GKVK-5.*

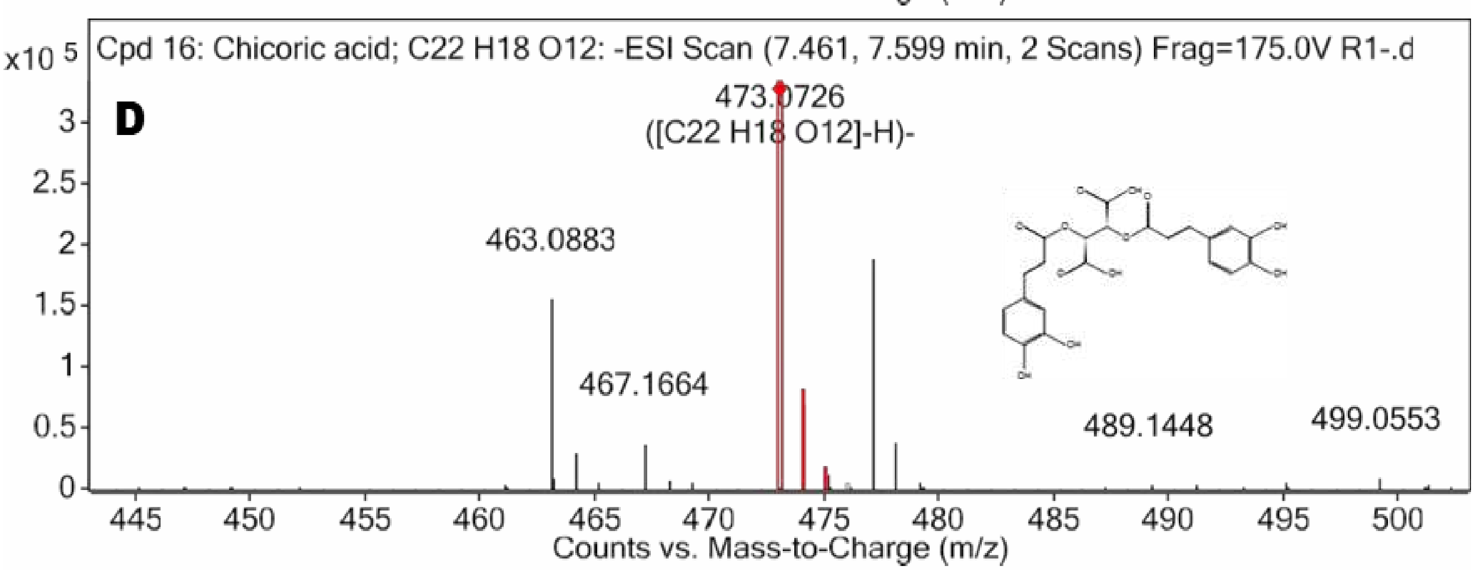




Cpd 12: Astragalin 7-rhamnoside; C27 H30 O15: -ESI Scan (6.042, 6.256 min, 2 Scans) Frag=175...
x10 5
B
593.1510
([C27 H30 O15]-H)-
569.1977
599.2071
Counts vs. Mass-to-Charge (m/z)



Cpd 15: Rutin; C27 H30 O16: -ESI Scan (7.247, 7.382 min, 2 Scans) Frag=175.0V R1-.d
x10 5
C
609.1457
([C27 H30 O16]-H)-
597.1814
617.1978
635.1581
Counts vs. Mass-to-Charge (m/z)



Cpd 16: Chicoric acid; C22 H18 O12: -ESI Scan (7.461, 7.599 min, 2 Scans) Frag=175.0V R1-.d
x10 5
D
473.0726
([C22 H18 O12]-H)-
463.0883
467.1664
489.1448
499.0553
Counts vs. Mass-to-Charge (m/z)

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