한작지(Korean J. Crop Sci.), 56(1): 94~98(2011)

Proximate Chemical Composition and Endogenous Gibberellins of Chufa (*Cyperus esculentus* L.) in Upland and Wetland

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ABSTRACT Proximate chemical components (protein, oil, carbohydrate, ash, fiber, and starch) were determined from tubers grown in upland and wetland conditions. The contents of crude protein, oil, carbohydrate, and starch were higher in upland condition than in wetland condition. Eight gibberellins were commonly identified and quantified in leaves and tuber of chufa grown in wetland and upland field during growing season. Gibberellin content was always higher specifically in the leaves and tubers grown in wetland condition than in those grown in upland condition. The current knowledge of gibberellin biosynthesis suggests that the two endogenous bio-active gibberellins both GA_1 and GA_4 are differently metabolized according to cultural conditions. Major gibberellin biosynthesis route is ascertained dominantly the non C-13 hydroxylation pathway leading GA_4 in chufa plants.

Keywords : proximate chemicals, gibberellins, tuber, chufa, wetland, upland, *Cyperus esculentus*

Chufa (*Cyperus esculentus* L.) is a member of Cyperaceae family is one of the finest nuts from the tropics to the temperature regions. It is one of the oldest cultivated plant species in the North America and grown spontaneously all over Turkey (Mokady and Dolev 1970, Oderinde and Tairu 1988, Coskuner *et al.* 2002). The plant prefers sandy, loamy and clay soils and can grow in heavy clay soil. It grows commonly in seasonally flooded wetlands. It is considered to be superior oil that compares favorably with olive oil. The tubers contain up to 30% of non-drying oil, it is used in cooking and in making soap. Tubers have got the typical aroma of almond and nut. In the chemical composition of tubers, there are 20.1 to 41.7% starch, 20.9 to 30.2% oil, 10.6 to 20.2% sugar, and 5.1 to 15.1% crude fiber. The chufa was first introduced late 1980's in Korea. There were few studies on the evaluation of higher tuber production referring moist-soil management, stressed providing food, starch properties and forage crop (Sung *et al* 2004, Lee and Hwang 2002). In respect to agronomic performance to increase tuber yield and growth of ground part, it is necessary to compare growing conditions of chufa plants in two different wetland and uplands in order to determine optimal cultural practices with proximate chemical components. The present investigation was carried out to find the proximate composition, growth properties of chufa tubers, and endogenous gibberellins in wetland and upland fields.

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MATERIALS AND METHODS

Chufa tubers purchased from the local farm market in Canada were used. Chufa tubers were commonly planted on a hill-seeded with the planting distance 30 cm between plants within rows and 20 cm between rows on ridges on 1 June in wetland and upland. Prior to planting, fertilizer was supplied with nitrogen, phosphorus, and potassium at the rate of 85, 25, and 70 kg ha^{-1}, incorporating as basal and top dressing (7:3, w/w) to the soil. Irrigation was kept intermittently to 2 cm from the soil surfaces every 20 days in wetland. Experimental design was a randomized complete block with three replicates. Protein, ash and fiber contents of tubers were determined by the methods (AOAC, 2005). Starch content was also measured according to Abdel-Aker and Michalinos (1963). Chufa tuber was crushed and pressed by hydraulics laboratory press for oil extraction. The extracted were dried over anhydrous sodium sulphate, and filtered through Whatman No. 1 filter and then weighed for determining oil content. For gibberellin analysis, the tubers harvested were immediately frozen in liquid nitrogen and stored at -80℃. When all the required materials for GA analysis had been collected, the samples were lyophilized for 48 h. The extraction of endogenous gibberellins was followed as described by Lee *et al.* (1998). The GAs were chromatographed on a 3.9×300 mm μBondaPak C_{18} column (Waters, USA) and eluted at 1.5 ml min^{-1} with following gradient: 0 to 5 min, isocratic 28% MeOH in 1% aqueous acetic acid; 5 to 35 min, linear gradient from 28 to 86% MeOH; 35 to 36 min, 86 to 100% MeOH; 36 to 40 min, isocratic 100% MeOH. Up to 50 fractions of 1.5 ml each were collected. Small aliquots (15 μl) from each fraction were taken, and radioactivity was measured with liquid scintillation spectrometry (Beckman, LS 1801, USA) to determine accurate retention times of each GA based upon the elution of ^{3}H-GA standards. The fractions were dried on a Savant Speedvac and combined according to the retention times of ^{3}H-GA standards and previously determined retention times of the labeled (deuterated) GA standards. GAs were quantified using [17, 17-$^{2}H_2$]-GAs (20 ng each) as internal standards (purchased from Prof. L. N. Mander, Australian National University, Canberra, Australia). The five prominent ions were analyzed by GC-MS-SIM (Finnigan Mat GCQ) with dwell times of 100 ms. Endogenous GA contents were calculated from the peak area ratios respectively. Retention time was determined by the hydrocarbon standards to calculate the KRI value (Kovats, 1958). The standard deviation was calculated using Sigma plot 2001 software (Jandel Scientific, San Rafael, CA, USA).

RESULTS AND DISCUSSION

Growth characteristics and tuber production of chufa plants grown in wetland and upland were estimated (Table 1). It was clear that chufa grown in wetland showed increased plant height, tuber number and tuber yield as compared to the chufa grown in upland. In characteristics of tubers, width and length of tubers were more promoted in wetland than in that of upland, although number of tuber per plant was significantly decreased in wetland as compared to the upland.

Higher tuber yield grown in wetland resulted from the increased tuber width and length. Chufa can withstand temporary flooding if the aerial part of plants was not completely submerged under water and prolonged flooding during the growing season is generally not recommended in chufa cultivation. However, maximum tuber production was occurred in wetland.

Table 2 shows the chemical composition of the chufa tubers grown in wetland and upland. Protein content in both wetland upland ranged 4.0 to 4.4% showing higher percentage in chufa tuber grown in upland. Oil content was significantly higher in chufa tuber grown in upland compared to the chufa grown in wetland. High values of carbohydrate founded in the chufa tubers grown in upland as to be 47.9% of tuber dry weight. Carbohydrates were

Table 1. Changes of growth characteristics of tuber in upland and wetland grown chufa (*Cyperus esculentus* L.).

Field condition	Plant height (cm)	Fresh tuber Weight (g)	Tuber width (mm)	Tuber length (mm)	Tiller (no/plant)	Tuber (no/plant)	Tuber yield (kg/ha)
Upland	74.6	0.32	18.5	18.3	54.7	89.4	9,891b
Wetland	85.4	0.44	22.2	22.3	30.4	101.5	15,063a

The same letters in the columns are not significantly different at 5% level by DMRT.

Table 2. Changes of chemical composition in upland and wetland grown chufa (*Cyperus esculentus* L.) tubers.

Field condition	Protein (%)	Oil (%)	Carbohydrates (%)	Ash (%)	Fiber (%)	Starch (%)
Upland	4.4a	31.1a	47.9a	4.3a	6.5a	22.7b
Wetland	4.0b	28.7b	45.3b	4.3a	6.6a	25.3a

The same letters in the columns are not significantly different at 5% level by DMRT.

also the most abundant in the tubers. Ash content was not changed by growing field conditions. Fiber content was slightly increased in wetland. However, it did not show significant difference in growing conditions.

The chufa tubers grown in upland condition had high amounts of starch compared to the chufa tubers grown in wetland. It was reported that proximate compositions analyzed from Nigerian tigernut (chufa) were protein 8.0%, fat 24.3%, fiber 24.0%, ash 1.8%, and carbohydrate 30.0%, respectively (Ekeanyanwu and Ononogbu, 2010). Compared with this literature, the contents of protein and carbohydrate in two growing conditions were relatively low and high in ash content. It is referred that tiger nut and chufa species can change the chemical contents due to different ecological habit characters.

Endogenous gibberellins, GA_{53}, GA_{19}, GA_{20}, and GA_1, and five were members of the non-C13-hydroxylation pathway, GA_{12}, GA_{24}, GA_9, and GA_4, were only identified and quantified in the chufa tubers by a comparison of their mass spectra and Kovats retention indices (KRI) with these from a spectral library of Gaskin and MacMillan (1991) (Table 3). Three endogenous gibberellins, GA_{15}, GA_{44}, and GA_{36}, were not identified.

Figure 1 shows changes of bioactive gibberellin (GA_1 and GA_4) contents in tubers of chufa grown in wetland and upland. Bioactive GA_4 content in tubers grown in wetland was the highest but bioactive GA_1 level was the lowest at initial tuberization stage (Jul 10). Bioactive GA_1 level was only highest at Jul 20 regardless of growing conditions, otherwise, bioactive GA_4 content was also highest values at Jul 30 independent of growing conditions. The only bioactive GA_4 content of chufa plant grown in wetland maintained high levels during growing seasons.

We have been compared the bioactive endogenous gibberellin contents (GA_1 and GA_4) from leaves of chufa plants grown in upland and wetland (Fig. 2). Bioactive GA_1 content was

Table 3. GC-MS analysis of HPLC fractions from acidic ethyl acetate fraction of the chufa (*Cyperus esculentus* L.) tubers.

HPLC fraction	GAs	KRI†	Source	*m/z* (%, relative intensity of base peak)‡				
37-38	GA_{53}	2450	sample	448(46)	251(30)	235(30)	389(25)	241(18)
		2450	standard	450(46)	253(27)	237(27)	391(25)	243(18)
43-46	GA_{12}	2335	sample	300(100)	240(31)	328(21)	360(2)	285(19)
		2335	standard	302(100)	242(29)	330(20)	362(2)	287(20)
39-40	GA_{15}	-	sample	-	-	-	-	-
		2608	standard	239(100)	284(28)	344(23)	312(22)	298(13)
26-28	GA_{44}	-	sample	-	-	-	-	-
		2789	standard	207(100)	432(63)	238(41)	417(12)	373(17)
34-36	GA_{24}	2444	sample	314(100)	226(89)	286(77)	342(42)	374(4)
		2444	standard	316(100)	228(88)	288(76)	344(43)	376(4)
29-31	GA_{19}	2600	sample	434(100)	374(59)	402(41)	462(10)	375(57)
		2600	standard	436(100)	376(55)	404(42)	464(11)	377(53)
37-38	GA_9	2305	sample	298(100)	270(78)	227(49)	243(43)	330(7)
		2305	standard	300(100)	272(76)	229(50)	245(44)	332(5)
24-25	GA_{20}	2485	sample	418(100)	375(45)	403(14)	359(12)	301(13)
		2485	standard	420(100)	377(40)	405(14)	361(12)	303(12)
26-28	GA_{36}	-	sample	-	-	-	-	-
		2600	standard	284(100)	430(58)	312(47)	462(11)	402(39)
32-33	GA_4	2506	sample	284(100)	225(80)	289(70)	224(76)	418(27)
		2506	standard	286(100)	227(78)	291(68)	226(75)	420(26)
12-14	GA_1	2674	sample	506(100)	448(20)	313(17)	491(13)	377(12)
		2674	standard	508(100)	450(18)	315(16)	493(14)	379(11)

†KRI, Kovats retention indices.
‡Gibberellin was identified with five ions and quantified by first ion with comparison of labeled standards.

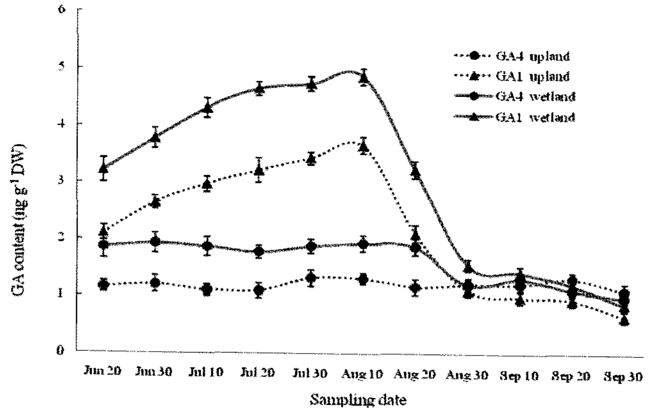

Fig. 1. Changes of bioactive gibberellin GA_1 and GA_4 content in tubers of chufa (*Cyperus esculentus* L.) grown in upland and wetland during growing season. Error bars show standard deviation (n=3).

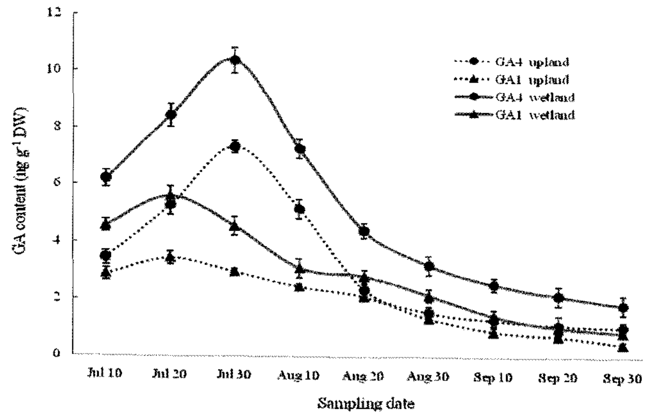

Fig. 2. Changes of bioactive gibberellin GA_1 and GA_4 content in leaves of chufa (*Cyperus esculentus* L.) grown in upland and wetland during growing season. Error bars show standard deviation (n=3).

increased slightly until Aug 10, and it was sharply decreased by Aug 20 but then fell. Meanwhile, bioactive gibberellins (GA_4) showed a lower level and proceed to Aug 20 compared to the GA_1 during growing season. Bioactive gibberellins (GA_1 and GA_4) remained constant from Aug 30 to Sep 30. It was reported that endogenous gibberellin (GA_1) was induced and increased by prolonged submergence in leaves of *Rumex palustris* (Joris *et al.*, 2006). Furthermore, this suggests that gibberellin 3-oxidase may be dominantly involved in the conversion of GA_{20} to GA_1 because the GA_{20} level is similar to the GA_1 level. It is recognized that gibberellins are involved in seed development and their increase is known to stimulate seed and tuber maturity

deviation (n=3).

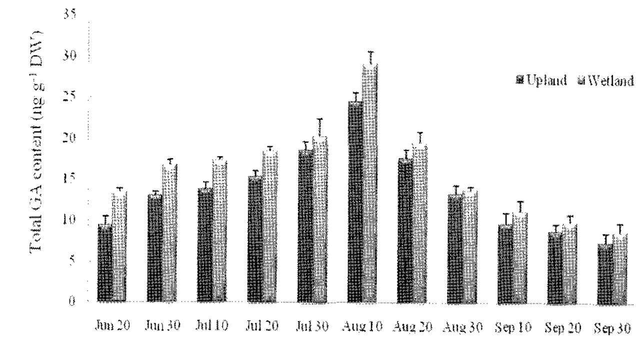

Fig. 3. Changes of total gibberellin contents in tubers of chufa (*Cyperus esculentus* L.) grown in upland and wetland during growing season. Error bars show standard deviation (n=3).

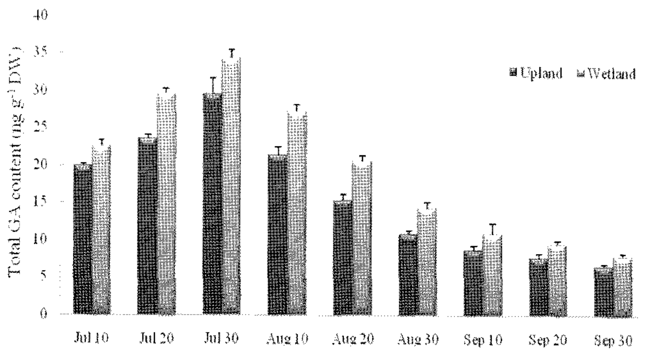

Fig. 4. Changes of total gibberellin contents in leaves of chufa (*Cyperus esculentus* L.) grown in upland and wetland during growing season. Error bars show standard deviation (n=3).

(Swain *et al.* 1997).

Changes of total gibberellin contents in tubers of chufa plants grown in wetland and upland has shown in Figure 3. Total endogenous gibberellins content in tuber during growth season ranged 9 to 30 ng. Gibberellin content was gradually increased until Aug 10 in both wetland and upland and then decreased. Gibberellin content in tubers of chufa grown in wetland was always higher than that of upland. There was similar report that gibberellin (GA_4 rather than GA_1) is indispensable for promoting in tuber initiation and development (Kim *et al.*, 2003).

Figure 4 represents the alteration of total endogenous gibberellin contents in leaves of chufa grown in different field conditions. Total endogenous gibberellins were linearly increased to Jul 30 and its content was decreased after

Aug 10. Higher gibberellin contents in leaves of chufa grown in wetland were kept during growing seasons. Finally, it is interesting that gibberellin level was always higher in the leaves and tubers of wetland grown chufa than in those of upland grown chufa. The current knowledge of gibberellin biosynthesis suggests that the two endogenous bio-active gibberellins both GA_1 and GA_4 are specifically metabolized by different cultural conditions. Major gibberellin biosynthesis route was dominantly non C-13 hydroxylation pathway leading GA_4 in chufa plants.

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