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BIOLOGY

Chia (*Salvia hispanica*) experiment at a 30° N site in Sichuan Basin, China

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ABSTRACT: *The mysterious ancient Mesoamerican Indian crop chia (Salvia hispanica) is revived and expanding worldwide due to its richness of valuable nutraceuticals such as α-linolenic acid (ALA), antioxidants, food fiber, gels, and proteins. We carried out a pilot experiment on chia planting in non-frost Sichuan Basin, at Hechuan Base (30˚0' 43" N, 106˚7' 41" E, 216 m), Southwest University, Chongqing, China. The split-plot trial contained two factors, 3 spring-summer sowing times as main plots, and 6 densities as subplots, with 3 replicates. Phenological, botanical, adversity, yield, and seed quality traits were investigated. Plants were very tall, suffered from lodging, and flowered in mid-October. Sichuan Basin can be considered as a north edge for growing chia, with low yield (680 kg/hectare) because of insufficient seed filling and maturation in autumn-winter season (1000-seed weight of 1.14 g). However, its ALA content is 5 percent points higher than the seed-donor commercial bottle (65.06%/63.96% VS 59.35%/59.74% for black/white seeds), accompanied by decrease oleic and stearic acid, while linoleic acid and palmitic acid are equivalent. Considering its short-day habit, it is recommended to try sowing in middle summer (from late June to early August) to avoid too long growing period, excessive vegetative growth, and waste of field and climate resources caused by spring-summer sowing. Furthermore, winter sowing of chia with mulch cover could also be tried, with an expectation of harvesting in summer. Most importantly, only when the photoperiod-insensitive early flowering stocks are created, chia can be recommended as a low-risk crop to the farmers of this region.*

Key words: *chia (Salvia hispanica), field trial, Chongqing, yield, α-linolenic acid (ALA).*

Performance agronômica da chia (*Salvia hispanica)* cultivada aos 30° N na Bacia de Sichuan, China

RESUMO: *A chia (Salvia hispanica) é cultivada em todo o mundo por sua riqueza de nutrientes nutracêuticos valiosos, tais como a-ácido linolênico (ALA), antioxidantes, fibras alimentares, géis e proteínas. Entretanto, não há informações sobre sua performance agronômica se cultivada aos 30˚N* na China. Assim, realizou-se um experimento com o cultivo de chia na base *Hechuan (30°0'43"N, 106°7'41"E, 216m, que não apresenta geada) da Southwest University, Chongqing, China. O delineamento em parcela subdividida contém dois fatores,três épocas de semeadura na primavera-verão como parcelas principais e seis densidades de sementes como subparcelas, com três repetições. Foram investigados os caracteres fenológicos, botânicos, de adversidade, rendimento e qualidade da semente. As plantas se tornaram altas, acamarame floresceram em meados de outubro. A bacia de Sichuan pode ser considerada como uma fronteira limítrofe norte para o crescimento da chia, com baixo rendimento (680kg ha^{-1}) devido ao enchimento e amadurecimento insuficientes na estação outono-inverno (peso de 1000 sementes de 1,14g). No entanto, o seu conteúdo de ALA é de 5 pontos percentuais mais elevado do que a semente comercial, 65,06%/63,96% contra 59,35%/59,74% para as sementes pretas/brancas, respectivamente, acompanhado por diminuição de ácido oleico e ácido esteárico, enquanto que o ácido linoleico e o ácido palmítico são equivalentes. Considerando o seu hábito de dia curto, recomenda-se semear no meio do verão,de junho a início de agosto, para evitar um tempo de cultivo muito longo, desenvolvimento vegetativo excessivo e desperdício de recursos de campo e clima causados pela semeadura de primavera-verão. Além disso, a semeadura de inverno da chia com cobertura morta também poderia ser realizada, com expectativa de colheita no verão. Mais importante ainda, somente quando os estoques de floração precoce insensíveis ao fotoperíodo* são criados, pode-*se* recomendar como uma cultura de baixo risco para os agricultores desta região.

Palavras-chave: *chia (Salvia hispanica); ensaio de campo; Bacia do Sichuan; rendimento; aácido linolênico.*

INTRODUCTION

Nowadays, trends of eating style of modern people are changing depending on their individual preferences for food safety and for healthier food derivatives in which animal fat is replaced by vegetable oil sources more in line with nutritional recommendations. *Salvia hispanica* L., commonly known as chia, is an oil-rich annual herbaceous plant of the Lamiaceae (mint) family, and has been consumed and domesticated as a staple food crop by Mesoamerican Indian tribes since 2600 B.C. (AYERZA & COATES, 2005b; BOCHICCHIO et al., 2015b). Its fruits were one of the four main food

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sources traditionally used in pre-Colombian times, and with recently renewed interest and research undertakings, this ancient noble crop has again emerged as a new potential superfood and become a rediscovered "new" crop (AYERZA & COATES, 2009). Because of its high contents of poly-unsaturated fatty acids, anti-oxidants, vitamins, minerals, and protein in seeds, it can be widely used for many kinds of cookie recipes and industrial products for food and health-promoting purposes besides its traditional seed and leaves consumption (CAHILL, 2004; AYERZA & COATES, 2009; PEIRETTI, 2010). The literatures mentioned that chia seed contains an excellent source of ω-3 PUFAs (58-64% of total lipids), high content of protein (16-24%) and lipids (31-35%), and high level of fiber (34-56%) (SOSA et al., 2016). The oil contained in chia seeds in high percentage (>30%) is the richest natural source of ω-3 fat, alpha-linolenic acid (ALA), which is the sole precursor for biosynthesis of the physiologically crucial PUFAs (SDA, EPA and DHA) in human body after its ingestion from food sources (AYERZA & COATES, 2002).

Chia plants can grow very well in sandy, well-drained soils with a low nutrient content, moderate salinity, and soil pH of 6-8.5 (YEBOAH et al., 2014), but low soil nitrogen content seems to strongly reduce yield (COATES, 2011). Chia can be successfully grown under irrigated or rain-fed conditions, and grows very well with 300-1,000 mm rainfall during growing season. The optimal for chia growth is a well-distributed rainfall during vegetative growth and reproductive growth, and dry condition during seed maturation and harvesting (COATES & AYERZA, 1996; YEBOAH et al., 2014). The duration of crop cycle in most cases ranges from 140 to 180 days (COATES & AYERZA, 1996; VASTOLA, 2015), but since chia is extremely sensitive to photoperiodic day length (a macro-thermal short day species), the growing cycle absolutely depends on the latitude where it is planted (COATES, 2011). Chia cannot produce seeds when the temperature is very low (AYERZA & COATES, 2005b), and the minimum and maximum growth temperatures of this crop are 11˚C and 36˚C respectively, with an optimum range between 16˚C and 26˚C.

Though chia was one of the five staple crops of Aztec and many other Mesoamerican ancient kingdoms, it was the only crop failed in introduction from Mesoamerica to the whole world, though many other staple crops (corn, potato, sweet potato, tomato, hot pepper, tobacco, upland/sea island cottons, peanut, sunflower, pumpkin, common bean, papaya, etc.) were successfully introduced to the world right after the discovery of the New World by Cristoforo Colombo in 1492 (AYERZA & COATES, 2005a). The basic reason is that chia has strictly crucial short photoperiod habit, and it is relatively less tolerant to cold too (JAMBOONSRI et al., 2012). Its introductive planting in high-latitude regions like Spain did not flower in time in summer season, and plants died soon after budding in autumn due to early frosts. After its rediscovery by Dr. Coates at about 30 years ago, this crop is currently rapidly extending its introductive experiment sites and tentative planting regions from traditional Mesoamerican belt to quite a few new regions worldwide (SOSA et al., 2016; ORONA-TAMAYO et al., 2017), e.g. Argentina (BUSILACCHI et al., 2013), Chile (BAGINSKY et al., 2016), Brazil (DE FREITAS et al., 2016; DA SILVA et al., 2017), Bolivia (AYERZA, 2016), USA (JAMBOONSRI et al., 2012), Australia (TIMILSENA et al., 2017), India (SREEDHAR et al., 2015), Ghana (YEBOAH et al., 2014), south Italy (BOCHICCHIO et al., 2015b), etc. Nowadays chia is commercially cultivated for 370,000 hectares in several agricultural regions worldwide in 2014, mainly in Bolivia, Paraguay, Argentina, Mexico, Australia, Central America, Peru, Ecuador, and Colombia (SOSA, 2016; ORONA-TAMAYO et al., 2017). AYERZA & COATES (2009) have studied the crop in different areas in Central America, but research results on agronomic techniques for chia are still few (BOCHICCHIO et al., 2015a). However, because of photoperiod sensitivity, the feasible geographic belts for cultivating traditional chia germplasms for grain production is restricted to 22°55'N-25°05'S (HILDEBRAND et al., 2013), and at higher latitudes the probability of the crop reaching maturity is low (AYERZA & COATES, 2005a). In recent years, scientists begin to create artificial photoperiod-insensitive germplasms for extending chia cultivation belts to subtropical and even temperate regions. Through EMS and γ-ray mutation technologies, JAMBOONSRI et al. (2012) demonstrated chia mutant stocks, which were able to flower with a photoperiod 15h in greenhouse and a photoperiod of 14h 41min in the field. A photoperiod-insensitive chia variety 'Sahi Alba 914' was selected from a field natural mutant and patented (SORONDO, 2014), but it is not yet a commercial variety.

However, to date, there is no report on chia planting and chia study in China, though China is the largest country in view of Agriculture in the world. This paper reporting on pilot introductive experimental performance is the first chia research in China. For a new crop being introduced, different aspects of plant population and spatial arrangement need to be understood. Sowing date is an extremely relevant variable of crops in determining the growth and development durations in response to environmental clues. According to a previous report, the north edge for growing traditional chia can be extended to basin valleys near 32˚N in USA (JAMBOONSRI et al., 2012). Valleys in Sichuan-Chongqing basin of China is around 30˚N and have no frost, so we chose an experimental site (30˚0′43″N, 106˚7′41″E) in Hechuan District, Chongqing City (the central province of southwest China) to test whether in China this similar condition is also the north-edge for chia planting. The two-factor experiment aims to test the flowering and seeding possibility, agronomic and yield performance, and quality compositions of chia at this speculated north edge.

MATERIALS AND METHODS

Experimental site

The study was conducted during the season from March to December in 2016. The experiments were set up in Caobachang research field of Hechuan Base, Experimental Farm of Southwest University, Weituo Town, Hechuan District, Chongqing City, China. The site is located at latitude 30˚0′43″N and longitude 106˚7′41″E, with an altitude of 216 meters. Over the course of the year, the temperature typically varies from 43˚F to 94˚F and is rarely below 37˚F or above 103˚F. The hot season lasts for 2.7 months, from June 16 to September 7, with an average daily high temperature above 85˚F. Cool season lasts for 3.2 months, from November 25 to March 1, with an average daily high temperature below 60˚F. The wet season lasts 6.6 months, from April 2 to October 20. The dry season lasts 5.4 months, from October 20 to April 2.

Experimental design and field demarcation

The experiment was conducted using a split-plot design with three replications. The main plot treatment was three sowing times (S1=March 30, S2=April 13, and S3=May 24 in 2016) and the subplot treatment was sowing densities at six levels (row spacing x plant spacing) as D1=62.5cm x 25cm (1 plant hole^{-1}), D2= 50cm x 25cm (1 plant hole^{-1}), D3=41.66cm x 25cm (1 plant hole^{-1}), D4=62.5cm x 25cm (2 plants hole^{-1}), D5=50cm x 25cm (2 plants hole^{-1}), D6=41.66cm x 25cm (2 plants hole^{-1}), thus (D1=64,000 plants/ha, D2=80,000 plants/ha, D3=96,000 plants/ha, D4=128,000 plants/ha, D5=160,000 plants/ha and D6=192,000 plants/ha) respectively. Planting interval between first and second (15 days), and second and third sowing times (41 days) were different due to severe drought in April and May because chia experiments were conducted under rain fed conditions. A field measuring is 135m x 8m for 3 replicates (8m x 2.5m per replicate). Direct sowing in the field was done and seeds were spread in the drills and covered lightly to enhance germination. After sowing, the land was immediately covered by mulching plastic film not only for maintaining moisture to encourage germination but also to control the weed growth throughout the growing season. Chia (*Salvia hispanica* L.) accession from commercial cultivar of The Chia Co of Australia was sown with natural rainfall (without irrigation). Soil tillage consisted of ploughing and harrowing (two times) at 35cm depth, and the crop was hand-weeded by three times.

Investigation and data collection

During growing period, branching architecture, inflorescences, longitudinal section of bud, immature seeds, mature white seed, stem cross-section were investigated. The plots were harvested by hand on 28 December 2016 from a total of (S1=273, S2=259 and S3=218) days old plants, respectively. Plants from each plot were harvested, and the seed yield and yield components were counted (plant yield, plant height, stem diameter, branch number, ear number, height of 1st branch, full seed of top ear, empty seed of top ear and 1000 seed weight) on 10 plants per sub-plot, and the yield of the chia product, consisting of dry indehiscent fruits commonly called seeds, was determined on a fresh basis. Ratio of full to empty fruits was determined on a subsample of top ear per plant on 10 plants per plot where fruits were manually separated based on color and degree of filling. After harvest, the seed heads were threshed, and the seed were cleaned and weighed. The moisture content was decreased to constant after oven drying at 35ºC for 3 days. Besides, during growing period, weeds, diseases, pests, and lodging performance were investigated and identified accordingly if they appeared.

Gas chromatography (GC) analysis of seed fatty acids profile

All seeds from each plot were hand harvested and cleaned. A sub-sample from each treatment and each replication was obtained, and random 200mg of each black and white seed sample was taken from each sowing batch to conduct laboratory analysis. Commercial Australia chia seed from The Chia Co were used as control. 200mg of mature chia seeds was used to synthesize the FA methyl ester (FAME), and the FAMEs were analyzed on a Gas Chromatograph Model GC-2010 (Shimazu, Japan) according to the published method (LIAN et al., 2017). Peaks were identified by typical retention time of each FA composition in chia seed oil. Results were determined by using area percentage of FAME in total detectable peak areas.

Statistical analysis

The data collected were subjected to analysis of variance by using Statistical 8 Software and mean comparison were done by Least Significant Difference (LSD) at 5% and 1% levels. Pearson's correlation was calculated.

RESULTS AND DISCUSSION

Phenological and botanical performance of chia in Chongqing

Direct sowing (ca. 10 seeds/hole) in the field was done. With plastic covering, the chia seeds gradually germinated 4-5 days after sowing, and one week later most seedlings came out. True leaves came out two weeks after sowing. First thinning (5 seedlings/hole) was carried out two weeks after sowing, and final thinning to the designed densities was done two months after sowing. Transplanting for missing seedlings was done 3 weeks after sowing. The Hechuan Base chia research field (Figure 1A), stem cross-section (Figure 1B), and chia branching architecture (Figure 1C) are presented.

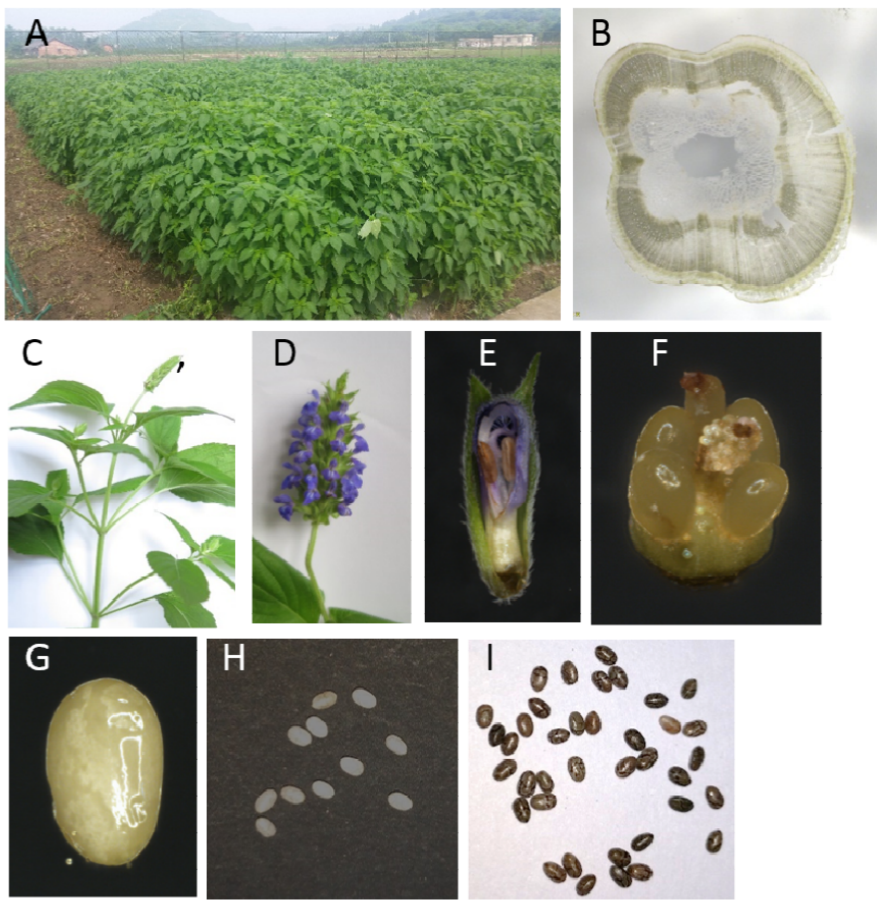

Figure 1 – Field and botanical traits of chia plant cultivated in Chongqing. A, field overview; B, stem cross-section; C, chia branching architecture and young ears; D, inflorescence at full blossom; E, longitudinal section of large bud; F, semi-mature seed; G, immature seeds; H, mature white seed; I, mature dark seed.

For all treatments, plants showed little difference in budding time and flowering time. Bud initiation started in September last week, and the inflorescence (Figure 1D) could be seen in October first week. On Oct 15 opening of the first flower could be seen in the field, but the blossoming date of the whole field was as late as the end of October. The maturation procedure of early-batch seed started in the early of November, but the seed on the middle and upper positions of the ear developed and grew much more slowly because of temperature dropping into cold condition after mid-November. On the harvesting day (Dec 28), only lower-position seeds were fully mature, the mid-position seeds were semi-mature, and the upper-position seeds were small and immature (Figure 1E-I).

More strategies for growing traditional short-day chia in Sichuan Basin could be explored. Sowing in spring and early summer, like in this experiment, should not be recommended, because this will result in too long growing period, excessive vegetative growth, and waste of field and climate resources. Sowing in middle summer (from late June to early August) could be tried, but how to ensure germination and early seedling growth in heat and drought field should be considered, e.g. irrigation, intercropped with summer crops, etc. Furthermore, since the low valleys here have an average temperature of about 9ºC in January (the coldest month), winter sowing of chia with mulch cover could be tried, which perhaps allows chia plants to pass through short photoperiod in spring, flower in early summer, and seed might be harvested in summer. How large the plants will grow, how dense they should be sown, and whether the yield and quality are acceptable, are three major concerns of these two strategies.

Creating early flowering novel germplasms of chia is urgent. Though University of Kentucky and company TFSB, LLC from USA have reported or patented early flowering or photoperiod-insensitive chia stocks/varieties, these are not enough and cannot be accessed without a contract. Considering the nutraceutical values and development potential of chia, our world needs many early flowering chia stocks, which could be efficiently created by artificial mutations.

Adversities of chia growth in Chongqing

Lodging

After 70 days old, plants of first growing batch started to fall down due to the overgrowth in high planting densities, since Sichuan Basin is typically warm and humid. After chia plants falling down, the upper stem restored upright growth habit soon, and nodes of the creeping stem section produced adventitious roots (Figure 2A). Almost all chia plants reached the maximum plant height after 3-4 months of sowing, and then started lodging. Lodging percentage of first sowing batch (S1= almost 6 months old) was 98.52%, second sowing batch (S2= almost 5 months old) was 96.32%, and third sowing batch (S3=almost 4 months old) was 94.92%. The lodging problem was mainly caused by over-growth of the too long vegetative duration since the plants could not reach flowering before October, thus the plant height values of all treatments were more than 2m or even nearly 2.5m. Previous chia studies reported plant heights of less than 2m (JAMBOONSRI et al., 2012; BUSILACCHI et al., 2013; YEBOAH et al., 2014; BAGINSKY et al., 2016; DE FREITAS et al., 2016; DA SILVA et al., 2017). We speculate there were three reasons: fertile field, heavy base fertilizer application, and too long vegetative growth duration. Besides, it was noticeable that, compared with other crops, the stem of chia was brittle and easy to be broken. In the future, it is worthy to test the contents and proportions of the three mono-lignin (G, H, and S types) and investigate the crossing styles of cell wall ingredients in chia stem.

Drought and heat stresses

In Sichuan Basin the summer season from June to early September is hot, accompanied by drought and heat stresses. This was very typical in 2016, since the highest day temperature from middle June to August 25 was at 30-40ºC in most days, and the corresponding lowest night temperature was also high with 25-30ºC in most days. In this duration, the total rainfall was insufficient and from July to August, drought and stresses got stronger and stronger. However, the chia plants survived this summer season, and showed considerable tolerance to longtime drought and heat stresses, though temporal wilting symptoms appeared (Figure 2B). Nine to eight irrigation times were needed per growing season in commercially chia growing areas, depending on climatic conditions and rainfall that could produce highest seed yield (AYERZ & COATES, 1996). Our results showed that chia can grow under natural rain-fed with tolerance to the strong summer hot and drought season.

Diseases

In our experiment, chia plants showed light affection by diseases in the whole growth season, thus no chemical was spayed to control diseases.

However, occasional diseases could be observed on a few plants, e.g. white rot stem wilting of some lodging plants, and ear rot by a red fungus (Figure 2C, D, E). The actual pathogen and pathogenesis need further investigation in the future.

Pests

Chia plants have distinct volatiles, which are believed to play crucial roles in repelling certain pests (VASTOLA, 2015). In our study, most chia plants were not distinctly fed by pests, but a kind of brown cutworm was very harmful to young chia seedlings (Figure 2F). In the daytime it hided in the soil, and in the night it came out and fed on the seedlings, especially cutting the hypocotyls. At adult plant stage, some other worms could be found eating a few chia leaves (Figure 2G, H), and some white flies, aphids, and ladybugs could also be reported at the later growing stage of chia. These pests could not reach the economic threshold level, so the plants were not sprayed with pesticides.

Weeds

Weeding time was few because, under dense planting, chia was early canopy closure, which could suppress weeds and reduce weed/crop competition, especially for sunlight, soil nutrients, and soil water. The following two types of weeds were found in chia growing area as a minor problem (Figure 2I, J, K). The seven types of superior weeds

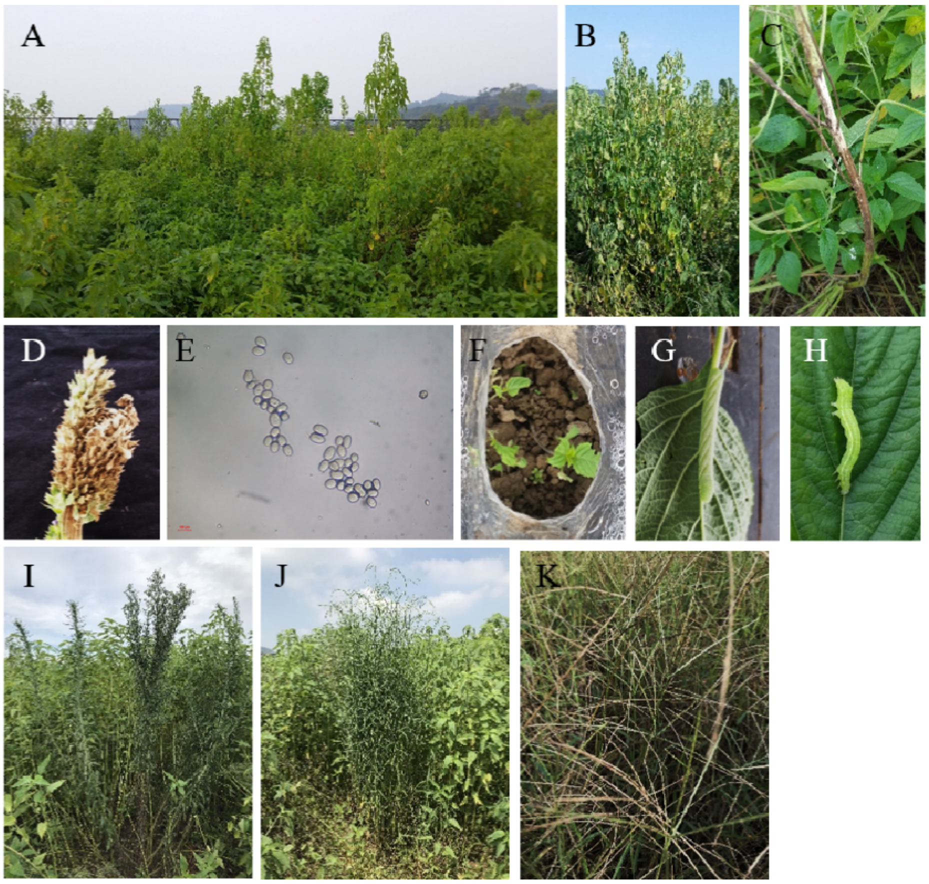

Figure 2 – Adversities of chia in Chongqing. A, lodging field on Oct 6; B, drought field on Aug 23; C, white rot stem wilting on Sep 21; D, ear rot on Dec 28; E, spores of the fungus causing ear rot; F, seedling symptom of cutworm damage; G, a tailed worm feeding on adult plant; H, a non-tailed worm feeding on adult plant; I, the typical weed *Erigeron canadensis*; J, the typical weed *Aster subulatus*; K, the typical weed *Digitaria sanguinalis*.

were *Phytolacca Americana* (pokeweed), *Lactuca indica* (Indian lettuce), *Aster subulatus, Erigeron canadensis* (or *Conyza canadensis* or *Canada fleabane), Broussonetia papyriferq* (paper mulberry), *Amaranthus hybridus* (green amaranth, slim amaranth, smooth amaranth, smooth pigweed, or red amaranth), and *Sambucus javanica* (Chinese elder); and the nine types of inferior weeds were *Polygonum perfoliatum* (Asiatic tearthumb), *Solanum fligrum, Miscanthus sinensis* (Chinese silver grass), *Eleusine indica* (Indian goosegrass), *Portulaca oleracea* (little hogweed or parsley), *Echinochloa crus-galli* (barnyard grass), *Digitaria sanguinalis* (hairy crabgrass), *Alfernanthera philoxeroides* (alligator weed), and *Leptochloa chinensis* (Chinese sprangletop).

Agronomic and yield performance of chia in Chongqing

Length of growing period (defined as planting date through harvest date), seed yield, and 1000-seed weights are presented in Table 1. Sowing time extremely significantly (*P<0.01*) influenced seed yield. Results of statistical analysis showed that the maximum seed yield (7.10g/plant) was received in sowing time S3, followed by S2 (5.12g/plant), and S1 (4.82g/plant) respectively. Differences between sowing time S3 & S1 and S3 & S2 were extremely significant (*P*<0.01) at three growing periods. No significant (*P*<0.05) difference in seed yield between sowing times S1 and S2 was recorded. This highest seed yield observation might be due to the relatively shorter length of growing period of S3 (218 days, VS S1 273 days and S2 259 days). The commercial chia growing area situated in different environmental conditions in low-latitude regions like Bolivia, Argentina, Mexico, and Ecuador, among which the critical growing periods are between 100–150 days that can produce the economically highest seed yield (AYERZA & COATES, 2009). This could be assumed that too long growing period observed in our experiment was non-optimal for setting up yield components, and enhanced loss by lodging problem. The most important factor influencing the yield assessments is the optimal growing period which can determine the best seed yield (ANDRADE et al., 2002). Results obtained for seed yield under different sowing times also agreed with the findings (PARK & REE, 1964; AYERZA & COATES, 1996; COATES, 2011) who reported that yield was affected by planting date. There was no irrigation facility in our experimental site (Hechuan Base), which was under natural rain fed conditions. Therefore, longer growing periods for S1 and S2 faced water shortage problem or moisture requirement problem for critical

Table 1 - Yield and 1000-seed weight of chia planted with three sowing times in Chongqing.

Treatment	Density (per hectare)	Plant grain (g)	Seed weight (g/1000 seeds)	yield (kg/hectare)
----------Sowing time----------				
S1 (273 days)	120,002	4.82b	1.16	578
S2 (259 days)	120,002	5.12b	1.10	614
S3 (218 days)	120,002	7.10a	1.17	852
LSD0.05 [2]		1.15	0.07	
----------Density----------				
D1	64,000	6.01	1.12	385
D2	80,000	5.07	1.12	406
D3	96,015	5.75	1.14	552
D4	128,000	5.96	1.14	762
D5	160,000	5.51	1.16	882
D6	192,000	5.70	1.17	1094
LSD0.05		1.63	0.11	
Pr>F Sowing time Density		<0.001** 0.86	0.18 0.88	
CV%		29.98	9.67	

S1, S2, S3: sown on Mar 30, Apr 13, May 24, respectively, all harvested on Dec 28 2016. D1 to D6: 6 densities from 64,000 to 192,000 plants per hectare. Means in a column within a group with the same letter are not statistically different (*P*<0.05). *significant different at 5% level. **extremely significant different at 1% level.

yield determination. The environmental factors such as soil moisture content and temperature strongly influenced the chia seed yield (YEBOAH et al., 2014). Chia seed yields reported herein ranged from 578 to 852 kilograms per hectare in three different sowing times. Although, yields were quite low, they were still in line with the production yield between 450 and 1,250 kilograms per hectare (400 to 1,120 lb/acre) reported for experimental plots sown in Argentina and Colombia (COATES & AYERZA, 1996; COATES & AYERZA, 1998). Generally, variations in seed yield were observed among the sowing times showing that the trait was subjected to influence by the environment. There was no significant difference (P<0.05) in seed yield among different planting densities. Results contradict the finding of YEBOAH et al. (2014) who reported that under the planting density (40,000 plants ha^{-1} with narrow-row spacing (0.5m x 0.5m)) generated the highest yield. In our experiment, even the lowest planting density was 64,000 plants ha^{-1} with row spacing (0.625m x 0.25m), so the density levels in our experiment perhaps were all higher than optimal, and this was the reason of lodging problem and non-significant yield difference among densities.

No significant difference (P<0.05) in 1000-seed weights (1.10-1.17g) was reported among sowing times and planting densities. The 1000-seed weight produced by plants was influenced by both genetic and environmental factors. Therefore, using only one genotype (Australian) in all sowing times and planting densities could not affect 1000-seed weights distinctly. The 1000-seed weight of chia seeds we bought was 1.39g, indicating that the seeds we harvested in early winter at Sichuan Basin were significantly smaller. The cold temperature and lack of sunshine at middle to late stages of seed growth were strong limiting factors in our experiment.

The effect of sowing time and planting density on botanical and agronomic traits (plant height, stem diameter, branch number, ear number, height of 1st branch and full grain percentage) are presented in Table 2. Sowing time extremely significantly (P<0.01) influenced stem diameter, branch number, ear number and height of the 1st branch. Sowing time S3 consistently recorded higher stem diameter, branch number, and ear number; and lower height of the 1st branch. There was no significant difference (P<0.05) in stem diameter, ear number, and height of first branch between S1 and S2. The highest

Table 2 - Agronomic traits of chia planted in Chongqing.

Treatment	Plant height (cm)	Stem diameter (cm)	Branch number	Ear number	Height of first branch (cm)	Full grain percentage (%) [3]
---Sowing time---						
S1	229.47	1.43b	26.19c	62.64b	72.23a	65.89
S2	228.87	1.55b	30.87b	71.51b	68.35a	65.30
S3	235.84	1.85a	36.78a	99.88a	48.53b	60.37
LSD0.05	12.11	0.13	2.29	10.68	7.26	5.76
---Density---						
D1	241.61	1.66	30.7	78.02	66.77	68.91a
D2	231.24	1.56	31.27	73.83	65.51	59.93b
D3	230.54	1.62	32.87	84.39	61.51	59.66b
D4	228.22	1.62	30.97	79.17	62.31	65.82ab
D5	226.6	1.59	31.13	77.2	57.6	62.85ab
D6	230.16	1.60	30.74	75.46	64.51	65.95ab
LSD0.05	17.12	0.18	3.24	15.11	10.27	8.14
---Pr>F---						
Sowing time	0.44	<0.001**	<0.001**	<0.001**	<0.001**	0.18
Density	0.57	0.91	0.76	0.79	0.52	0.16
CV%	7.72	11.72	10.81	20.21	17.01	13.31

S1, S2, S3: sown on Mar 30, Apr 13, May 24, respectively, all harvested on Dec 28 2016. D1 to D6: 6 densities from 64,000 to 192,000 plants per hectare. Means in a column within a group with the same letter are not statistically different (P<0.05). *significant different at 5% level. **extremely significant different at 1% level.

stem diameter (1.85cm) was observed in S3 other than in S2 (1.55cm) & S1 (1.43cm). There was extremely significant difference (P<0.01) in branch number among three sowing times, and the highest branch number was in S3 (36.78), followed by S2 (30.87) and S1 (26.19). The highest ear number (99.88) was observed in S3 other than in S2 & S1. There was no significant (P<0.05) difference in ear number between S1 (62.64) and S2 (71.51). Sowing times S1 and S2 consistently produced higher first branch (72.23cm and 68.35cm) than S3 (48.53cm). The basic reason of these differences was that all the 3 sowing batches experienced too long duration of vegetative growth before flowering in October, early spring sowing suffered more from lodging, early organ senescence, summer high temperature and drought.

No significance (P<0.05) in full grain percentage was reported among 3 sowing times. As just a single cultivar was used herein; therefore, there was little variation in flowering time and full grain percent among sowing times. There was also no significant difference in plant height among sowing times. Seedlings of early sowing time S1 (March) and S2 (April) might face low temperature at the seedling stages which might cause slow seedlings growth because chia species does not have strong cold tolerance. Average minimum/maximum temperatures were 11.52/18.26 in March and 16.2/23.43 in April at our experimental site. Finally, the final plant heights of three sowing times would be similar because they all had a long vegetative growth duration in warm season, and the whole growing periods were much longer than the optimal growing period 100-150 days of chia in Bolivia, Argentina, and Ecuador (AYERZA, 2009).

There was no significant difference (P<0.05) in plant height and branch number among different planting densities. Results contradicted findings (YEABOAH et al., 2014) who reported that planting density significantly influenced plant height and branch number. In our experiment, planting density was too high, thus all chia plants got similar plant heights, branch numbers, stem diameter, ear number, and height of 1st branch among different planting densities. Among the density treatments, the full grain percent on top ear was highest in D1 (68.91%), followed by D6 (65.95), D4 (65.82), and D5 (62.85), while D2 (59.93%) and D3 (59.66) were significantly lower. The higher the density, the stronger the competition for light, water, minerals, etc., and the more serious of lodging influence.

Fatty acids profile of chia seed harvested in Chongqing

According to our gas chromatography test results (Figure 3; Table 3), chia seeds either from our experiment or from The Chia Co Australian contained α-linolenic acid (ALA, ω-3) as the major fatty acid, accompanied with considerable linoleic acid (LA, ω-6) and minor percentages of palmitic acid (PA), oleic acid (OA), and stearic acid (STA). The ALA, LA, OA, STA, and PA contents were 59.35%/59.74%, 21.47%/21.31%, 8.47%/8.31%, 3.51%/3.51%, and 7.20%/7.13% in the black/white chia seeds from Chia Co bottles, and were 65.06%/63.96%, 20.93%/22.94%, 5.11%/4.85%, 1.96%/1.89%, and 6.94%/6.35% in the black/white chia seeds from our experiment, respectively. Though PA proportions were a little higher, the overall fatty acids profiles in our test were similar to previous reports (COATES & AYERZA, 1996; COATES & AYERZA, 1998). It is interesting that chia seeds harvested in our experiment contained significantly higher ALA (65.06%/63.96% VS 59.35%/59.74% for black/white seeds) and extremely significantly lower OA (5.11%/4.85% VS 8.47%/8.31%) and STA (1.96%/1.89% VS 3.51%/3.51%) than those of the Australian source, while the LA (20.93%/22.94% VS 21.47%/21.31%) and PA (6.94%/6.35% VS 7.20%/7.13%) are similar. Since ALA is the featured and most valuable fatty acid of chia (AYERZA & COATES, 2005a; 2009; BOCHICCHIO et al., 2015a; ORONA-TAMAYO, et al., 2016; SOSA, 2016), it means that chia seeds harvested in Sichuan Basin in early winter have better quality as an ALA source than the commercial bottles bought from The Chia Co.

As for the reason for ALA increase and OA and STA decrease in chia seeds harvested in Sichuan Basin in early winter, cold tolerance adaptability stands out. Cold affects the transcription of fatty acid desaturases and oil quality in the fruit of oil olive (*Olea europaea* L.) (MATTEUCCI et al., 2011). In soybean, the *GmFAD3* family, and more specifically *GmFAD3A*, may play a role in the cold response (ROMÁN et al., 2012). Rice mutants deficient in ω-3 fatty acid desaturase (FAD8) fail to acclimate to cold temperatures (TOVUU et al., 2016). When an *Arabidopsis* chloroplast ω-3 fatty acid desaturase gene *FAD7* was introduced into tobacco, the transgenic plants showed enhanced tolerance to cold (KODAMA et al., 1994; KHODAKOVSKAYA et al, 2006). Though the summer in our experiment is hot enough, the seed filling stage of chia is from October to December. It is reasonable that the cool season with temperature declining will simulate the expression of gene(s) encoding ω-3 fatty acid desaturase (FAD3, FAD7, FAD8) to biosynthesize more ALA to improve cold tolerance.

All the fatty acids showed extremely significant differences (P<0.01) among 3 sowing

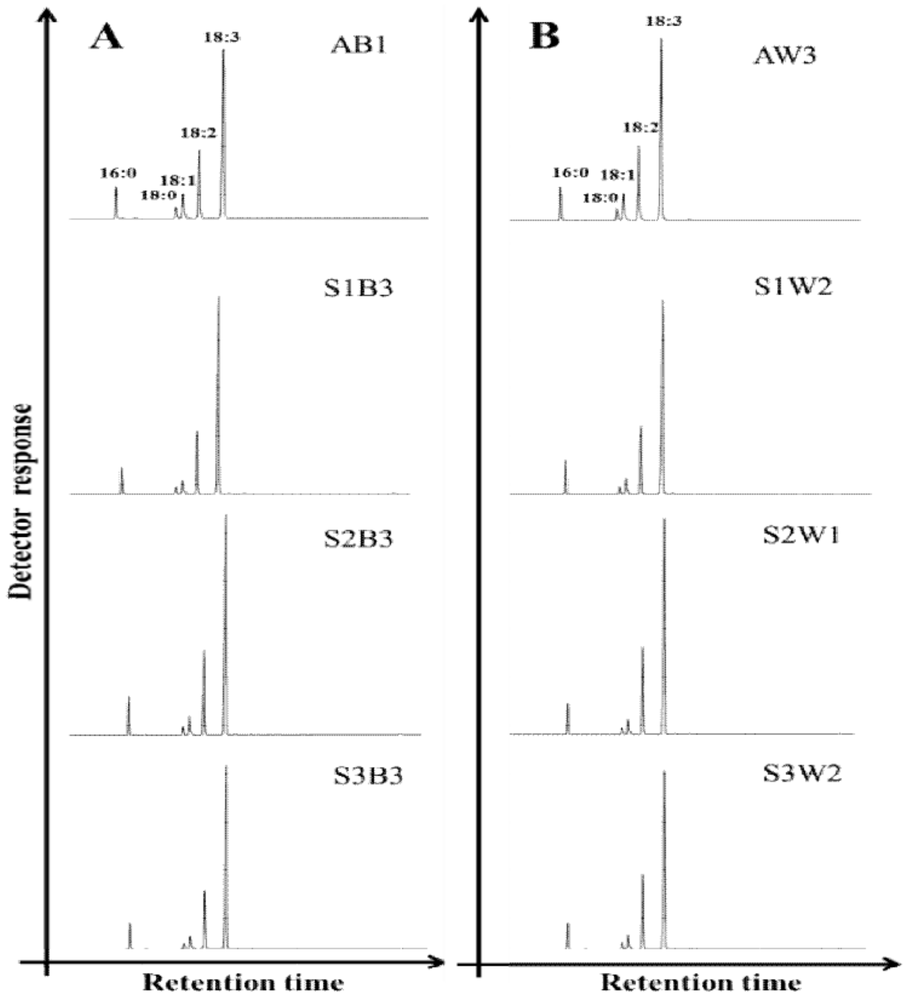

Figure 3 – Fatty acid GC profiles of chia seeds grown in Hechuan Base of SWU, Chongqing, China, with the seeds bought from The Chia Co Australia as a reference. 16:0, palmitic acid; 18:0, stearic acid, 18:1, oleic acid; 18:2, linoleic acid; 18:3, α-linolenic acid. AB1 and AW3: black and white seeds from The Chia Co Australia; S1B3, S2B3 and S3B3: black seeds harvested from 1st, 2nd and 3rd sowing treatments; S1W2, S2W1 and S3W2: white seeds harvested from 1st, 2nd and 3rd sowing treatments, respectively.

times, similar to COATES & AYERZA (1996) that oil content and fatty acid composition were affected by location and seeding date. Environmental factors (such as temperature, light, soil composition and type/variety) have variably significant effects on the nutrient composition of chia especially its fatty acid composition, oil content, and protein (DYBING & ZIMMERMAN, 1966; CHAMPOLIVIER & MERRIEN, 1996; AYERZA & COATES, 2004). Among the different fatty acids, LA showed the highest coefficient of variance (7.19% among sowing dates, and 7.8% between our experiment and bought seed), and CV% of other 4 kinds of fatty acids were relatively lower and similar (2.13-3.94%). Seeds of the first sowing time showed significantly higher ALA content than other sowing batches, but ALA had no strong correlation with sowing time course in this experiment. Similarly, there was no distinct rule for LA, OA, STA, and PA variations among sowing times. These could be explained by different growth conditions and lodging

Table 3 - Fatty acids contents of chia seeds grown in Hechuan Base of SWU, Chongqing, China, with the seeds bought from The Chia Co Australia as a reference.

Treatment	Name	Seed color	Palmitic acid (PA, %)	Stearic acid (STA, %)	Oleic acid (OA, %)	linoleic acid (LA, %)	α-linolenic acid (ALA, %)
S1B	1st sowing	black	6.70b	1.93ab	4.85bc	20.67b	65.85ab
S1W		white	6.60b	1.82b	4.74c	18.67b	68.16a
S2B	2nd sowing	black	7.13a	1.99a	5.56a	21.37b	63.95bcd
S2W		white	6.53b	2.03a	4.99b	25.37a	61.08d
S3B		black	6.98a	1.96a	4.93bc	20.7b	65.38abc
S3W	3rd sowing	white	5.93c	1.82b	4.81bc	24.79a	62.65cd
$LSD_{0.05}$			0.28	0.12	0.29	2.52	2.69
Pr>Ft			<0.001**	0.006**	0.002**	0.002**	0.005**
CV%			2.13	3.09	2.56	7.19	2.57
SB	Hechuan,SWU	black	6.94a	1.96b	5.11b	20.93a	65.06a
SW	Hechuan,SWU	white	6.35b	1.89b	4.85b	22.94a	63.96a
AB	The Chia Co	black	7.20a	3.51a	8.47a	21.47a	59.35b
AW	The Chia Co	white	7.13a	3.51a	8.31a	21.31a	59.74b
$LSD_{0.05}$			0.54	0.14	0.45	3.37	3.27
Pr>Ft			0.03*	<0.001**	<0.001**	0.53	0.01*
CV%			3.94	2.59	3.35	7.8	2.64

S1, S2, S3: sown on Mar 30, Apr 13, May 24, respectively, all harvested on Dec 28 2016. S=S1+S2+S3; A: seed from The Chia Co Australia (CK); B: black seed; W: white seed. Means in a column within a group with the same letter are not statistically different (*P*<0.05). *significant difference at 5% level. **extremely significant difference at 1% level.

influences among the sowing time treatments. This was also supported by the finding of AYERZA (1995) and COATES & AYERZA (1996) that there was a variation in fatty acid contents; although, using the common genetic sources due to the effect of different environmental conditions.

CONCLUSIONS

In this study, we carried out a pilot experiment on chia introduction and planting at 30ºN in the frost-free Sichuan Basin. Our preliminary results give the following conclusions. Sichuan Basin can be considered as a north edge for chia planting. In southern low valleys of this basin, chia plants sown in spring and summer can survive the strong summer heat and drought, get into reproductive growth in autumn and early winter, and certain amount of mature or almost mature seeds can be harvested. Traditional short-day chia grown in Sichuan Basin has low yield because of insufficient seed filling and maturation. Spring-sowing chia at here suffers from excessive vegetative growth, lodging, late flowering, partial mature and lower seed weight and yield, which does not hamper chia germplasm propagation but limits chia commercial productivity. Chia seeds bared in autumn-winter season in Sichuan Basin has better quality as an ALA source. On average its ALA content is about 5 percent points higher than the source seeds (65.06%/63.96% VS 59.35%/59.74% for black/white seeds), OA and STA decreases, while LA and PA are equivalent.

AUTHORS' CONTRIBUTION

The authors Aung Naing Win and Yufei Xue contributed equally to this work.

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DECLARATION OF CONFLICTING OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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