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Studies on Ashwagandha [*Withania somnifera*] Elite Genotypes for Root Yield and Attributes under Heat Stress Condition

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Abstract: Present investigation was carried out to assess the impact of environment on Indian ginseng (ashwagandha) root yield and its associated traits under semi-arid condition. Twenty-two elite genotypes of ashwagandha (*Withania somnifera*) were evaluated under normal and cold stress environment conditions for root yield and other associated traits. Analysis of variance, cold stress index and correlation, analysis were performed using the mean data. Analysis of variance revealed that the genotypes differed significantly under normal as well as under stress environment for all the plant traits studied. Best performing genotypes for root yield (kg/ha) under both conditions were HWS105 (433 kg/ha), HWS 153 (399 kg/ha), HWS1333 (396.3 kg/ha), HWS120 (372.4 kg/ha), HWS206 (308.1 kg/ha) and HWS 8-18 (304.1 kg/ha). Further, in correlation analysis, root yield had the significant positive association with root length and root diameter. Therefore, these characteristics can be exploited to improve dry root yield in ashwagandha genotypes and there is also scope for the selection of promising genotypes (based on the stress susceptibility index) from the present germplasm for cultivation under stress environment. Under stress condition, genotype HWS 153 exhibited good potential for commercial cultivation as well as it showed less reduction in root yield and low in SSI, but before recommendation for commercial cultivation to be tested over time and space.

Keywords: *Withania somnifera*, Ashwagandha, Environment stress, Root yield, Susceptibility Index

Indian is home of herbal medicines which are found in different hotspots including these three biodiversity centers i.e., the Himalayas, the Western Ghats, and the Indo-Burma. India covers about 11 percent of the global diversity of plants (Sakachep and Rai 2021). Ashwagandha (*Withania somnifera* (L.) Dunal (2n=48), Indian ginseng is one of the most valuable medicinal plant used in the Indian and Unani systems of medicine since ancient times (Kaileh et al 2007, Mishra 2014). The roots of ashwagandha have properties similar to ginseng roots; hence it is also known as Indian ginseng (Singh and Kumar 1998). It is also known as gooseberry or winter cherry and belongs to the family Solanaceae. The India, North-Western and Central partsin Africa, the Mediterranean region are the native palaces of ashwagandha. The most suitable climatic region for its growth and development is the dry and subtropical type. In India, it is mainly cultivated in the states of Madhya Pradesh, Rajasthan, Gujarat, Maharashtra, Punjab, and Uttar Pradesh. India is also an exporter of ashwagandha roots in the international market. According Zauba (2020), India sales maximum ashwagandha to USA of amounting about USD 883,780 followed by New Zealand (USD 101,606) and China (USD 90,552).

In Ayurvedic era, it was mainly consumed as a health tonic for fitness, longevity and vitality (Singh *et al.*2010).It is also very important stimulation of the human body immune system cells, phagocytes and lymphocytes, which also assist to manage the effects of stress and encourage wellness (Singh et al 2001, Singh et al 2003). Sirvastava et al (2018) observed that roots are also utilized to treat asthma, bronchitis, emaciation, dementia, insomnia, inflammation, neurological disorders, and Parkinson's disease. The total alkaloid content in the Indian ginseng roots vary from 0.13 to 0.31%, however, in some genotypes it also reported up to 4.3% (CSIR 1982; CCRUM 2007). The main chemical constituents are alkaloids and steroidal lactones. The medicinal properties of the plant are due to the presence of withanolides (withaferin A and withanolide D) (Sharma et al 2011). As per Indian chemotype, it has 12 types withanolides, five unknown alkaloids, several free amino acids, glycosides, tannins, chlorogenic acid, glucose, and several flavonoids in the leaves (Khare 2007). Earlier, the medicinal herbs and shrubs were available effortlessly in the nearby jungles and on mountains. Presently, due to constant utilization of these natural plants with alarming speed reduced their accessibility. Therefore, it is an insistent need to evolve the high yielding superior cultivars of essential medicinal flora to manufacture the superior quality raw drugs in order to meet out the local requirements of Ayurvedic practitioners as well as the international demands of medicine manufacturing agencies. Moreover, sufficient genetic diversity is available in this crop (Yadav et al 2008, Reddy et al 2012), and very little work has been done on its genetic improvement in spite of the long history of its domestication. A large portion of it still comprises wild, semi-wild plants or primitive cultivars which have not acquired genes for high productivity under cultivation and have great potential to improve the yield and quality. Medicinal plants are generally cultivated under harsh climatic conditions; therefore, identification of the superior genotypes for high temperature tolerance is essential for effective manipulation through breeding techniques (Yadav et al 2013). Therefore, keeping the above points in view, the newly developed elite genotypes of Ashwagandha were evaluated for heat stress under semi-arid conditions.

MATERIAL AND METHODS

To conduct the field experiment, 22 newly developed elite genotypes (Table 1) of Ashwagandha (*Withania somnifera* (L.) were grown in RBD on 7thSeptember 2017 (normal sown) during cropping season 2017-18 and on 28 October 2018 (late sown) during cropping season 2018-19 at Research Farm of MAP Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar (Haryana, India) located 29º10′ N latitude and 75º46′ E longitude with an elevation of 215.2m above the mean sea level. The plot size was 4.0 m x 1.2 m with spacing 30 cm x 10 cm. The fertility status oil of field soil was sandy loam in texture, medium in organic carbon (0.46 %), available nitrogen (141.0 kg/ha), available phosphorus (14.0 kg/ha) and available potassium (240.0 kg/ha). Weekly weather parameters data recorded from research area during 2017-18 and 2018-19 given in Figure 1 & 2. All the recommended package of practices was carried out to raise a good crop. Data were recorded on five randomly selected plants for plant height (cm), branches/plant, root length (cm), dry root yield (kg/ha). The roots were dug out 200 DAS, washed with fresh water, and dried in an electric oven up to 7-8% moisture content.

Statistical analysis: The analysis was carried out using Statistical Software, available online on CCS Haryana Agricultural University, Hisar website (Sheoran et al 1998). The pooled mean data of all the characters over the location were used to make the comparison. Heat susceptibility index (HSI) was calculated for root yield and other quantitative

Table 1. Elite genotypes with source and their morphological marker characters

Elite genotypes	Source	Morphological marker characters
HWS105	CCS HAU, Hisar	Plant type semi-erect, berry colour orange, root texture starchy, high seed yield
HWS108	CCS HAU, Hisar	Plant type erect, berry colour orange, root texture woody, medium seed yield
HWS116	CCS HAU, Hisar	Plant type semi-erect, berry orange, root texture woody, medium seed yield
HWS118	CCS HAU, Hisar	Plant type semi-erect, berry colour orange, root texture woody, high seed yield
HWS119	CCS HAU, Hisar	Plant type erect, berry colour orange, root texture woody, high seed yield
HWS120	CCS HAU, Hisar	Plant type semi-erect, berry colour orange, root texture woody, high seed yield
HWS121	CCS HAU, Hisar	Plant type erect, berry colour orange, root texture woody, high seed yield
HWS128	CCS HAU, Hisar	Plant type erect, berry colour yellow, root texture woody, high seed yield
HWS138	CCS HAU, Hisar	Plant type erect, berry colour red, root texture woody, low seed yield
HWS140	CCS HAU, Hisar	Plant type semi-erect, berry colour red, root texture woody, low seed yield
HWS206	CCS HAU, Hisar	Plant type semi-erect, berry colour red, root texture woody, medium seed yield
HWS219	CCS HAU, Hisar	Plant type semi-erect, berry colour red, root texture woody, low seed yield
HWS224	CCS HAU, Hisar	Plant type semi-erect, berry colour red, root texture woody, low seed yield
HWS229	CCS HAU, Hisar	Plant type semi-erect, berry colour yellow, root texture woody, high seed yield
HWS240	CCS HAU, Hisar	Plant type erect, berry colour red, root texture woody, low seed yield
HWS1320	CCS HAU, Hisar	Plant type semi-erect, berry colour red, root texture woody, low seed yield
HWS1321	CCS HAU, Hisar	Plant type erect, berry colour red, root texture woody, medium seed yield
HWS1333	CCS HAU, Hisar	Plant type semi-erect, berry colour red, root texture woody, medium seed yield
HWS 153	CCS HAU, Hisar	Plant type erect, berry colour orange root texture woody, high seed yield
JA134	Mandsaur, M.P.	Plant type semi-erect, berry colour orange, root texture woody, low seed yield
RAS-16	RAU, Bikaner	Plant type semi-erect, berry colour yellow, root texture woody, medium seed yield
HWS8-18	CCS HAU, Hisar	High in root yield, berry colour yellow orange, medium in plant height, high in Withanolide-A content

traits over high temperature stress (late sown) and non-stress environment (normal sown) (Fisher and Maurer 1978). HSI = [l-YD/YP]/D Where, YD = mean of the genotypes in stress environment. YP = mean of the genotypes under non-stress environment. D = 1-[mean YD of all genotypes/mean YP of all genotypes].

RESULTS AND DISCUSSION

Plant height: The average plant height of both the years was maximum (116.1cm) in HWS1321 followed by HWS116, HWS219, HWS128, HWS8-18, HWS224, HWS229, HWS138, HWS108 and HWS140 (96.3 cm). Above finding were in agreement with the recent studies in terms of variation with earlier researchers (Mishra 2014, Yadav et al 2008, Dubey 2010).

Number of branches/plant: On the basis of average of both the years, the number of branches per plant was maximum in HWS120) followed by HWS119 HWS219, HWS224, HWS1321, HWS140, HWS229, HWS8-18, HWS118, HWS1320 and RAS-16. Similar variation was also obtained with different genotypes (Mishra 2014, Yadav et al 2008, Dubey 2010).

Root length (cm): On the basis of average of both the years, thelongest root length (cm) was observed in HWS 153 (67.5) followed by HWS105, HWS8-18, HWS1320, HWS121, JA134, HWS120, HWS1333, HWS128, HWS116 and HWS108. Mishra (2014), Yadav et al (2008) and Dubey (2010) also evaluated different genotypes of Ashwagandha for root length and reported significant variations.

Root diameter (cm): On the basis of average of both the years, themaximum root diameter (cm) was observed in HWS1333 (3.9 cm), followed by HWS120, HWS206, HWS138, HWS1321, HWS105, HWS118, HWS219, HWS224, HWS229 and HWS240 (2.7 cm).

Root yield (kg/ha): The yield was maximum in HWS105 (433 kg/ha), followed by HWS 153, HWS1333, HWS120, HWS206, HWS8-18, HWS121, HWS1320, HWS118, HWS138 and HWS140 (247.9 kg/ha).

Percent reduction in seed yield under stress: Heat stress imposes challenges for crops and has deleterious effects on the morphology, physiology, and reproductive growth of plants. High-temperature stress at the time of the reproductive stage is becoming a severe limitation for economic yield as the cultivation expands to warmer environments and increase in temperature variability due to climate change (Arya et al 2014, 2020). However, behavior of different genotypes under the heat stress was not uniform; some genotypes were adversely affected while the other genotypes were able to combat the stress. In present investigation, some genotypes exhibited the lower percent reduction in root yield which were JA134 (10.13 %) followed by RAS-16, HWS240, HWS229, HWS 153, HWS224, HWS1321, HWS119, HWS120, HWS108, HWS1320, whereas, some genotypes exhibited high percent reduction in root yield which were HWS8-18 followed by HWS128, HWS140, HWS116, HWS1333, HWS206, HWS219, HWS105, HWS138, HWS121, HWS118.

Heat susceptibility index: Heat susceptibility index (HSI) is also an important tool to identify the heat tolerant genotypes (Suresh et al 2018). In the present investigation, minimum HSI was in JA134 (0.36) and closely followed by RAS-16, HWS240. The other genotypes HWS229, HWS 153, HWS224, HWS1321, HWS119, HWS120 and HWS108 exhibited relatively higher. Therefore, the genotype JA134 and RAS-16 considered heat tolerant and may be utilized in crop improvement programme for the development of heat stress tolerant cultivars. The genotypes, HWS8-18, HWS128, HWS140, HWS116, HWS1333, HWS206, HWS219, HWS105, HWS121, HWS138, HWS1320 and HWS118 were susceptible to heat stress. Research on high temperature tolerance or susceptibility and its association with root yield and other related traits would help in the development of heat tolerant genotypes (Yadav et al 2013). High temperature stress at later stages, may adversely affect photosynthesis, respiration, water relations and membrane stability, and also modulate levels of hormones and primary as well as secondary metabolites (Arya et al 2014). Furthermore, throughout plant ontogeny, enhanced expression of a variety of heat shock proteins, other stress-related proteins, and production of reactive oxygen species (ROS) constitute major plant responses to heat stress (Wahid et al 2007). In order to cope with heat stress, plants implement various mechanisms, including maintenance of membrane stability, scavenging of ROS, production of antioxidants, accumulation and adjustment of compatible solutes, induction of mitogen-activated protein kinase (MAPK) and Ca-dependent protein kinase (CDPK) cascades, and, most prominently, chaperone signaling and transcriptional activation. All these mechanisms, which are regulated at the molecular level, may also be responsible for JA134 (0.36) and RAS-16 (0.39) genotypes to thrive under heat stress.

Correlation analysis: The results of studies on characters association of ashwagandha root yield and contributing traits is presented in. The present study revealed that under normal conditions, root yield was significantly associated with root length and root diameter. Likewise, under stress condition, root yield was significantly associated with root length and root diameter (Table 3). However, under stress

Table 2. Mean performance of root yield and its contributing traits during 2017-18 & 2018-19

Genotype	Plant height (cm)			No. of branches /plant			Root length (cm)			Root diameter (cm)			Root yield (kg/ha)		
	2017-18	2018 - 19	Mean	2017 - 18	2018-19	Mean	2017-18	2018-19	Mean	2017-18	2018-19	Mean	2017-18	2018-19	Mean
HWS105	110.0	79.2	94.6	4.3	3.7	3.9	59.7	43.6	51.6	2.8	2.8	2.8	506.6	359.4	433.0
HWS108	112.0	80.6	96.3	4.3	3.4	3.8	38.0	27.7	32.9	2.3	2.3	2.3	262.2	189.7	226.0
HWS116	120.0	86.4	103.2	3.0	2.3	2.7	38.3	28.0	33.1	2.2	2.1	2.2	256.2	176.1	216.2
HWS118	105.3	75.8	90.6	4.7	3.6	4.2	35.3	25.8	30.5	2.8	2.7	2.8	293.3	211.1	252.2
HWS119	110.0	79.2	94.6	8.0	6.2	7.1	35.0	25.6	30.3	2.5	2.5	2.5	262.5	192.4	227.4
HWS120	109.7	79.0	94.3	9.3	7.3	8.3	39.7	29.0	34.3	3.6	3.6	3.6	432.0	312.8	372.4
HWS121	101.0	72.7	86.9	3.3	2.6	2.9	40.7	29.7	35.2	2.5	2.5	2.5	308.7	221.8	265.2
HWS128	117.3	84.5	100.9	3.3	2.7	3.0	38.3	28.0	33.2	2.4	2.2	2.3	279.4	187.2	233.3
HWS138	113.3	81.6	97.5	3.7	2.9	3.3	30.3	22.1	26.2	3.2	3.2	3.2	291.2	209.2	250.2
HWS140	112.0	80.6	96.3	5.3	4.2	4.7	36.0	26.3	31.1	2.7	2.6	2.6	294.8	201.0	247.9
HWS206	106.0	76.3	91.2	3.7	2.9	3.3	34.0	24.8	29.4	3.6	3.4	3.5	363.1	253.2	308.1
HWS219	120.0	86.4	103.2	5.7	4.4	5.0	29.7	21.7	25.7	2.9	2.8	2.8	255.5	180.0	217.7
HWS224	115.0	82.8	98.9	5.5	4.3	4.9	28.5	20.8	24.7	2.8	2.8	2.8	235.1	174.8	205.0
HWS229	115.0	82.8	98.9	5.0	3.9	4.5	30.0	21.9	26.0	2.8	2.9	2.8	249.3	190.5	219.9
HWS240	108.3	78.0	93.2	4.0	3.1	3.6	33.7	24.6	29.1	2.6	2.8	2.7	262.5	202.7	232.6
HWS1320	106.7	76.8	91.7	4.7	3.6	4.1	43.0	31.4	37.2	2.4	2.3	2.3	304.4	219.4	261.9
HWS1321	135.0	97.2	116.1	5.5	4.3	4.9	29.0	21.2	25.1	2.9	3.0	2.9	252.3	187.4	219.8
HWS1333	82.5	59.4	71.0	3.0	2.3	2.7	39.0	28.5	33.7	4.0	3.8	3.9	468.0	324.6	396.3
HWS 153	77.5	55.8	66.7	4.0	3.1	3.6	78.0	56.9	67.5	2.0	2.0	2.0	456.3	341.6	399.0
JA134	70.3	62.6	66.5	4.7	3.1	3.9	36.7	32.2	34.4	2.2	2.2	2.2	158.0	142.0	150.0
RAS-16	83.7	65.8	74.8	3.3	4.6	4.0	28.7	33.6	31.1	1.9	1.5	1.7	178.4	159.2	168.8
HWS8-18	110.0	89.2	99.6	4.7	4.1	4.4	38.0	40.4	39.2	2.1	2.4	2.2	376.4	231.8	304.1
Mean	107.0	77.9	93.3	4.7	3.8	4.3	38.3	29.3	34.0	2.7	2.7	2.7	307.0	221.3	266.2
CD (5%)	9.25	3.62		0.42	0.39		3.32	3.15		NA	NA		26.3	23.5	
CV(%)	4.66	3.09		6.22	5.87		7.44	6.87		68.4	60		12.4	11.5	

Table 3. Correlation analysis of ashwagandha root yield and contributing traits

Characters	Environment	Root yield	Plant height	Number of branches/ plant	Root length	Root diameter
Root yield	Normal	1.000	-0.122 NS	0.035 NS	0.674**	0.432*
	Stress	1.000	-0.288 NS	0.039 NS	0.690**	0.429*
Plant height	Normal	-0.122 NS	1.000	0.257 NS	-0.404 NS	0.159 NS
	Stress	-0.288 NS	1.000	0.239 NS	-0.497*	0.142 NS
Number of branches/ plant	Normal	0.035 NS	0.257 NS	1.000	-0.122 NS	0.212 NS
	Stress	0.039 NS	0.239 NS	1.000	-0.153 NS	0.183 NS
Root length	Normal	0.674**	-0.404 NS	-0.122 NS	1.000	-0.230 NS
	Stress	0.690**	-0.497*	-0.153 NS	1.000	-0.312 NS
Root diameter	Normal	0.432*	0.159 NS	0.212 NS	-0.230 NS	1.000
	Stress	0.429*	0.142 NS	0.183 NS	-0.312 NS	1.000

* Significant at 5% probability level
** Significant at 1% probability level
NS = Non-Significant

condition, plant height was also significantly negatively correlated with root length.The earlier findings also revealed the significant association of root yield with root length, root diameter; however, they also reported significant correlation of root yield with plant height and number of branches per plant. In the present study, the plant height and number of branches per plant were not significantly associated with root yield (Yadav et al 2008, Dubey 2010, Mishra 2014). The present study was made to assess the root yield of elite genotypes under normal and heat stress environment for the identification of high yielding genotypes for semi-arid regions. The genotypes JA134 and RAS-16 were identified as heat tolerant. A drastic reduction was noticed in high root yielding genotypes whereas very less reduction was noticed

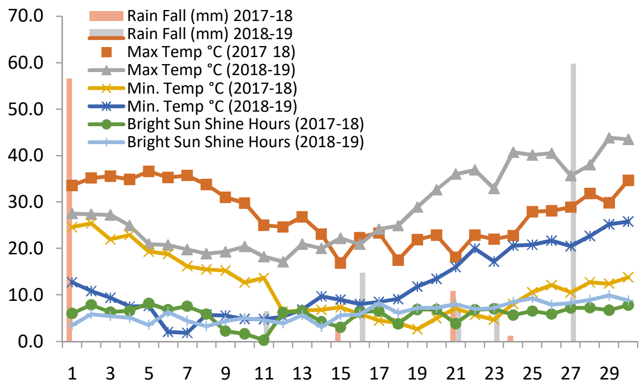

Fig. 1. Weekly weather parameters data recorded at Hisar during cropping season 2017-18 & 2018-19

A. Indian Ginseng growth under normal environmental condition

B. Indian Ginseng growth under stress environmental condition

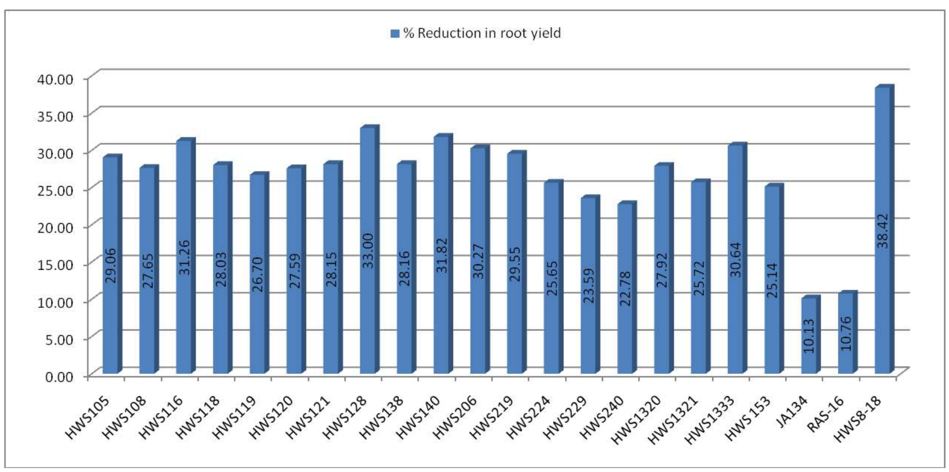

Fig. 2. Percent reduction in root yield in ashwagandha elite genotypes due to heat stress at Hisar

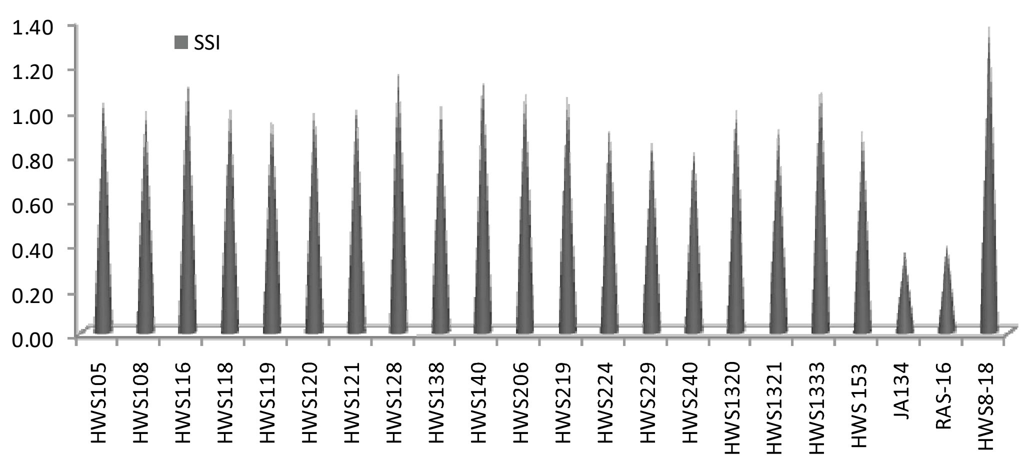

Fig. 3. Stress susceptibility index in seed yield in ashwagandha elite genotypes due to heat stress at Hisar

in poor yielding genotypes. It is concluded from the present study that genotypes HWS 105, HWS 1333, HWS 153 and HWS 120 are suitable for normal as well as heat stress conditions. Although, the percent reductions in root yield under stress in above genotypes varied from 25 to 30%. Even after such a reduction in root yield, they were also top performer in heat stress condition also. The ashwagandha genotypes, JA 134 and RAS-16 were low yielder but exhibited least percent reduction in root yield as well as lowest in HSI. Therefore, breeding among high yielding genotypes (HWS 105 and HWS 1333) and stress tolerant genotypes (JA 134 and RAS-16) may lead to development of transgressive segregants of ashwagandha, having the potential of high root yield as well as heat tolerant characteristics.

CONCLUSION

Best performing genotype for root yield (kg/ha) under both conditions wasHWS105 (433 kg/ha). To improve dry root yield in ashwagandha genotypes and there is scope for the selection of promising genotypes (based on the stress susceptibility index). Under stress condition, HWS 153genotype performed good potential for cultivation. It revealed less reduction in root yield and low in SSI, but before recommendation for commercial cultivation to be tested over time and space.

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