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天麻素的产地和品种分布特征及影响因子分析

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摘　要:文献收集近十年来野生和栽培天麻的活性成分(天麻素为主)、产地、品种、环境因子信息,采用数据统计探索天麻素在不同产地和品种样品中的分布特征;分析主要环境因子对天麻素累积的影响及不同活性成分间的相关性。结果表明:野生品天麻素含量(0.63%)高于栽培品(0.47%),贵州可能为最佳产区;栽培品以贵州(0.65%)、湖南(0.66%)、重庆(0.66%)为佳;纯种高于杂交品种,以绿天麻(0.57%)最优。年降水量对天麻素影响最大,最热月均温影响最小;天麻素与对羟基苯甲醇中等负相关,与巴利森苷 A、B 中等正相关。本研究可为天麻质量控制和合理栽培提供一定参考。

关键词:天麻;天麻素;产地;品种;环境因子;活性成分

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Analysis on Distribution Characteristics and Influencing Factors of Gastrodin According to Origins and Varieties

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Abstract:This article collected the active ingredients (Gastrodin-based),origins,varieties,environmental factors information of wild and cultivated *Gastrodia elata* nearly 10 years,and investigated the distribution characteristics of gastrodin in different producing areas and varieties by means of statistical data to analyze the effects of major environmental factors on the accumulation of gastrodin and the correlation between different active ingredients. The results showed that Gastrodin (0.63%) of wild *Gastrodia elata* was higher than the cultivar (0.47%),and Guizhou was the best wild area. The cultivated *Gastrodia elata* of Guizhou (0.65%),Hunan (0.66%),Chongqing (0.66%) was good. The purebred was higher than the hybrids ones,and *Gastrodia elata* f. viridis (0.57%) was the best. Annual precipitation had the greatest impact on gastrodin,and the effect of the hottest monthly mean temperature was the least;Gastrodin was negatively correlated with p-hydroxybenzyl alcohol and positively correlated with parishin A and B. This study can provide some reference for quality control and reasonable cultivation of *Gastrodia elata*.

Key words:*Gastrodia elata* Bl;gastrodin;origins;varieties;environmental factors;active ingredients

天麻来源于兰科植物天麻 *Gastrodia elata* Bl. 的干燥块茎,为传统名贵药材,具有息风止痉,平抑肝阳,祛风通络的功效;多用于高血压,高血脂,头痛眩晕,半身不遂等疾病的治疗[1]。现代研究表明,天麻素(Gastrodin)、对羟基苯甲醇(4-Hydroxybenzyl alcohol)、香草醇(4-Hydroxy-3-methoxybenzyl)、对羟基苯甲醛(p-Hydroxybenzaldehyde)、巴利森苷(Parishin)以及天麻多糖均具有较强的药理活性[2-5]。天麻药材野生资源较少,栽培品多以红天麻(*G. elata* Bl. f. elata)、黄天麻(*G. elata* f. flavida)、绿天麻(*G. elata* f. viridis)、乌天麻(*G. elata* f. glauca)和乌红杂交天麻(Hybrid *Gastrodia*)为主[6-8]。由于产地、品种、环境因子、种植技术等因素的差异,天麻中活性成分的含量参差不齐。目前,天麻中有效成分得到了广泛的研究,这些研究为天麻质量控制提供了一定的参考,但由于研究条件、样品采集等因素各不相同,导致研究结果存在较大差异。因此,通过查阅近十年的文献,筛选天麻中不同活性成分的含量数据及对应的产地、品种、环境因子特征,结合多种常用的数据挖掘方法,探索天麻素在不同产地和品种样品中的分布特征;分析主要环境因子对天麻素累

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积的影响效果及不同活性成分之间的相关性，以期为天麻的质量控制和合理栽培提供一定的参考。

1 数据采集

活性成分及样品信息数据采集于近十年的天麻质量研究相关文献[9-81]，环境因子数据来源于人地系统主题数据库（http://www.data.ac.cn/index.asp）和中国科学院资源环境科学数据中心（http://www.resdc.cn）。数据整理项包括：产地、品种、野生/栽培、环境因子（气候、土壤、海拔、年均温、最冷月均温、最热月均温、日照时长、年降水量、无霜期）及活性成分含量（主要为天麻素，部分项含有对羟基苯甲醇、巴利森苷A、巴利森苷B、巴利森苷C），不同产地样品情况见表1，不同品种样品情况见表2。

表1 不同产地样品情况

Table 1 Samples information of different producing areas

产地 Provinces	四川 Sichuan	云南 Yunnan	贵州 Guizhou	陕西 Shanxi	重庆 Chongqing	湖北 Hubei	安徽 Anhui	湖南 Hunan	西藏 Xizang	河南 Henan	甘肃 Gansu	东北 Dongbei	合计 Total
野生 Wild	2	11	9	0	11	0	0	0	9	0	0	0	42
栽培 Cultivation	69	96	76	119	19	76	51	35	11	9	18	15	594
合计 Total	71	107	85	119	30	76	51	35	20	9	18	15	636

表2 不同品种天麻样品情况

Table 2 Samples information of different varieties

品种 Varieties	红天麻 *G. elata* Bl. f. elata	黄天麻 *G. elata* f. flavida	绿天麻 *G. elata* f. viridis	乌天麻 *G. elata* f. glauca	杂交天麻 Hybrid *Gastrodia*	合计 Total
数据量 Data Quantity	156	10	11	83	26	287

2 数据处理

2.1 箱线图

箱线图（Box plot）利用最小值、上下四分位数、中位数、最大值五个统计量图形化后直观显示数据集的结构要点，可粗略表现数据的对称性，分布离散情况以及异常值等信息，具有较强的耐抗性，广泛应用于多组数据之间的比较[82]。

本文利用R软件中箱线图模块，对12个天麻产地和5个天麻品种中天麻素含量进行统计挖掘，箱体的长短、对称程度可显示不同产地、品种的天麻中天麻素含量数据分布特征。

2.2 随机森林模型对变量重要性评分

随机森林（Random forest）是一种较为新颖的基于分类树（Classification trees）的机器学习算法[83,84]。变量重要性评分（Variable importance measure）[85]是随机森林中对不同影响因子进行重要性评价的模块，基于排列（Permutation）随机置换的均方残差减小量（%Inc MSE）和模型精确度的减小量（Inc Node Purity）衡量相应自变量的影响力[86]，两个指标均通过主动给不同变量施加随机干扰，观察模型准确率的变化，根据准确率降低的幅度评价变量的重要性，即指标值越大，自变量对于因变量越重要，影响程度也就越高。与传统分类算法相比，具有速度快、准确性高等优点，且不用顾虑多元共线性问题，无需筛选变量，在医学、数据挖掘、生物信息学和生物生态等领域得到广泛应用[87,88]。

本文应用R软件对天麻素和环境因子共计636组数据进行随机森林回归，生成误差分布图，优选模型mtry值，并根据预测精度（Accuracy）、均方误差（Mean Squared Error，MSE）和置换检验的标准误差（Standard Errors）三个参数给出变量重要性直方图，筛选对天麻素含量有重要影响的环境因子。

2.3 相关性分析

相关性分析是指对两个或多个具备相关性的变量元素进行分析，从而衡量两个变量因素的相关密切程度。为更好度量两个随机变量的相关程度，引入了Pearson相关系数，其在协方差的基础上除以两个随机变量的标准差，取值范围-1到1之间，当两个变量的线性关系增强时，相关系数绝对值趋于1；当两变量无相关性或相关性极低时，相关系数绝对

值趋于0;“+”表示正相关,“示正表示负相关[89]。

本文筛选同时拥有天麻素、对羟基苯甲醇、巴利森苷A、巴利森苷B和巴利森苷C含量的文献数据并去除明显异常值,应用R软件中Performance Analytics模块进行可视化相关性处理,分析天麻中不同活性成分之间的相关性。

3　结果与分析

3.1　不同产地天麻素分布特征

将12个不同产地野生和栽培天麻中天麻素含量数据输入R软件进行数据挖掘,分布特征箱线图见图1。在总体636份样本中,96份样本数据低于0.2%,表明85%的样本符合药典标准[90]。据调查,野生天麻多分布在贵州、四川、西藏、云南、重庆五个产地,除西藏外,其他产地野生天麻中天麻素的含量均高于栽培品。野生样品均值为0.63%,中值为0.55%,均高于栽培品样本的0.47%和0.42%,以天麻素含量作为评判标准,野生天麻的质量明显优于栽培品。但栽培天麻中天麻素含量的方差、标准差、极差、四分位差分别为0.082、0.29、1.6%、0.36%,均小于野生品(0.24、0.48、1.96%、0.70%),显示野生天麻生长环境变化剧烈,个体差异较大,故栽培品质量更为稳定。

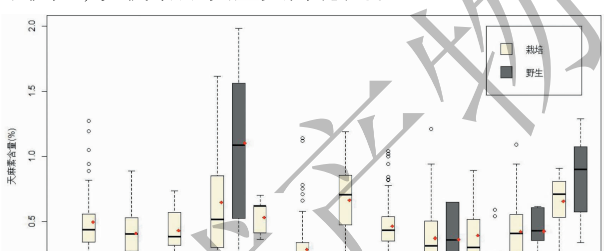


图1　天麻素产地分布特征

Fig. 1　Regional Distribution Characters of Gastrodin

注:红线表示2010版中国药典天麻素含量下限0.2%。红点表示均值

Note: The red line indicates the minimum amount of gastrodin in the Chinese Pharmacopoeia (2010 version) is 0.2%. The red dot indicates the mean.

贵州产野生天麻中天麻素的均值为1.10%,中值为1.09%,中值接近箱体中央,数据分布正态性强,含量最高。但是数据分布较为离散,表明该地区天麻个体差异较大。重庆产样品中天麻素均值为0.83%,中值为0.9%,四分位差为0.58%,无异常值,数据分布相对集中,天麻素含量较为稳定,质量较好。西藏产样品中天麻素含量最低,均值和中值分别为0.22%、0.14%,质量可能最差。

栽培品中,作为天麻传统产地的安徽、贵州、湖北、陕西、四川和云南地区天麻质量研究较多,样本含量均在50份以上,统计显示,贵州出产的天麻中天麻素含量较高,均值和中值都超过0.5%,但方差、标准差,极差和四分位差分别为0.25、0.5、1.93%、0.83%,数据分布离散,天麻个体差异较大。其余产地数据分布均较为集中,方差<0.07,标准差<0.3,四分位差<0.4,天麻质量稳定。湖北天麻的天麻素含量最低,均值和中值都低于0.3%,可能质量稍差。作为新发展产地的甘肃、河南、湖南、西藏、重庆和东北地区,天麻素数据分布较为集中,方差<0.07,标准差<0.26,四分位差<0.4%,质量较稳定。其中,安徽、湖南和重庆均值和中值都>0.5%,天麻素含量较高。西藏地区中值0.26%,均值0.32%,天麻素含量最低。

3.2　不同品种天麻素分布特征

收集红天麻、黄天麻、绿天麻、乌天麻和杂交天麻总共287份样品,其中有46份样本中天麻素含量低于0.2%,未达到药典规定标准。箱线图结果如图2所示。

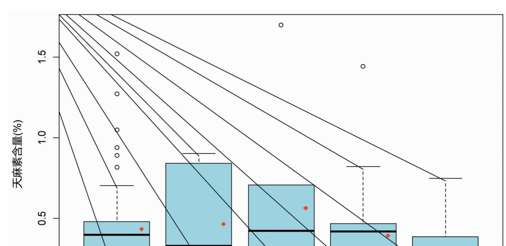


图2　不同品种的天麻素分布特征

Fig. 2　Distribution Characters of Gastrodin in different varieties

注:红线表示2010版中国药典天麻素含量下限0.2%。红点表示均值

Note: The red line indicates the minimum amount of gastrodin in the Chinese Pharmacopoeia (2010 version) is 0.2%. The red dot indicates the mean

不同品种天麻素含量差异明显,绿天麻中含量最高,均值和中值分别是0.57%、0.43%。红天麻、黄天麻、乌天麻样品中天麻素含量均高于药典标准。杂交天麻中天麻素含量的均值为0.25%,中值为0.19%,在这些品种中含量最低,部分样品的天麻素

含量不能达到药典规定标准。黄天麻和绿天麻中天麻素含量的标准差 >0.3,四分位差 >0.4%,数据分布较为离散,个体差异大。红天麻的标准差为 0.2,四分位差为 0.2%,表明红天麻数据分布最集中,质量最稳定。

3.3 随机森林模型对变量重要性评分

3.3.1 随机森林模型优化

预选变量个数(mtry)和随机森林中树的个数(ntree)分别为随机森林分析的两个重要参数。本文分别设置 mtry =2、3、4,ntree =500,建立随机森林回归模型,并生成误差分布图,结果见图 3。单棵树的错误率高于 18%,当 ntree >300 以后,误差线趋于平稳,而 mtry =2 时,错误率可达最低(13.4%)。故在该模型中选择 mtry =2、ntree =500 对变量进行重要性评分。

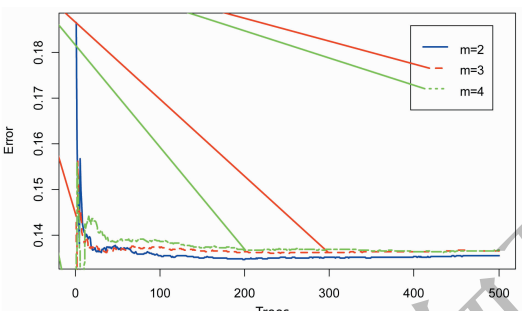


图 3 误差分布图

Fig. 3 Plot of Error distribution

3.3.2 环境因子变量重要性评分

基于最优的 mtry 和 ntree,变量重要性结果见图 4。以预测精度(Accuracy)为评价指标,年降水量评分最高,重要系数为 26.8,无霜期其次,重要系数 26.5,海拔重要性最低,重要系数为 18。以均方误差(MSE)为评价指标,年日照时数重要性最高,重要系数为 7.1,年降水量其次,重要系数为 7.0,最热月均温的评分最低,重要系数为 3.5。以置换检验的标准误差(Standard Errors)为指标,年日照时数重要性最高,但各因子无明显差异。综上,各环境因子对天麻素的影响程度依次可能为年降水量 >年日照时数 >无霜期 >最冷月均温 >年均温 >海拔 >最热月均温。

3.4 活性成分相关性分析

结合文献数据,对天麻中主要活性成分(天麻素、对羟基苯甲醇、巴利森苷 A、巴利森苷 B、巴利森苷 C)的含量进行相关性分析,结果见图 5。5 种成分含量均服从正态分布,天麻素和巴利森苷 A 正态性更强。天麻素含量与对羟基苯甲醇呈负相关(P <0.1),与巴利森苷 A(P <0.1)和巴利森苷 B(P <0.05)呈显著正相关。巴利森苷 A、巴利森苷 B、巴利森苷 C 的含量互相呈极显著正相关(P <0.01),天麻素与巴利森苷 C 无明显相关性,对羟基苯甲醇与巴利森苷 A、B、C 无明显相关性。

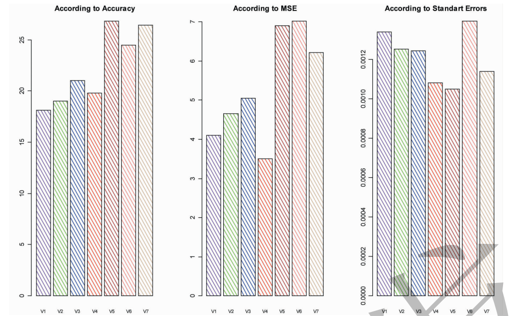
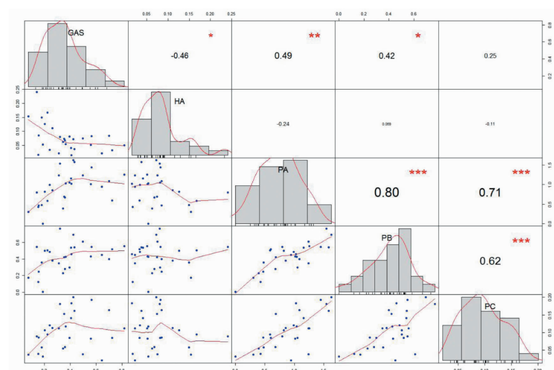


图 4 环境因子变量重要性图

Fig. 4 Importance of environmental factor variables

注:v1:海拔 v2:年均温,v3:最冷月均温,v4:最热月均温,v5:年降水量,v6:年日照时数,v7:无霜期

Note:v1:altitude,v2:annual temperature,v3:The average temperature of coldest month,v4:The average temperature of hottest month,v5:annual precipitation,v6:annual sunshine hours,v7:frostless period



注:G 表示天麻素,HA 表示对羟基苯甲醇,PA 表示巴利森苷 A,PB 表示巴利森苷 B,PC 表示巴利森苷 C。"+"表示正相关,"-"表示负相关;*在 0.1 水平显著相关,**在 0.05 水平显著相关,***在 0.01 水平显著相关

Note:G indicates Gastrodin,HA indicates 4-Hydroxybenzyl alcohol,PA indicates Parishin A,PB indicates Parishin B,PC indicates Parishin C. "+" Indicates positive correlation,"-" Indicates negative correlation;* Significant correlation at 0.1 level,** Significant correlation at 0.05 level,*** Significant correlation at 0.01 level

图 5 活性成分间相关性

Fig. 5 The correlation among different active ingredients

4 讨论

从本文分析结果可以看出,天麻素含量的变化幅度很大,且同时受到品种、产地、栽培、等级等因素影响,数据个体差异较大,为降低分析误差,在统计分析时,排除异常值对探索天麻素含量变化规律尤为重要。

比较天麻各产地生态环境可知,云南、贵州大部和川南一部分地区主要为横断山区和云贵高原,地势落差大,各产地气候差异显著,使得该片区天麻个体差异相对较大,样本数据分布更为离散。且该片区主要栽培品种为乌天麻,有研究表明,乌天麻在种植过程中受环境影响后常发生种内变异,在表型和遗传性上都不稳定[91],也是该片区天麻个体差异较大的原因。西藏地区海拔太高,长期处于低温状态,而天麻最适生长温度为 20 ~25 ℃[92],温度过低会使得天麻和蜜环菌生长受到抑制甚至发生冻害,使得西藏地区天麻样品天麻素含量低于其他产地。也有研究表明,矿质元素常决定天麻质量[93],如增施 $ZnSO_4$ 的天麻药效较对照大为提高,不同产地矿质元素差异较大,也使得不同产地天麻质量差异。

野生样品天麻素含量高于栽培品,与前人研究结果一致[94],但野生资源紧缺,故分析对天麻素积累有重要影响的环境因素,并在实际栽培中加以运用,提升栽培天麻质量才能满足现代市场的需求。

对比不同天麻品种中天麻素含量,绿天麻中天麻素含量最高,与刘小琴等[95]研究结果一致。目前绿天麻仅在南方及东北几个产地少量分布,资源相对紧缺,该品种的天麻值得研究推广。其次,黄天麻、红天麻、乌天麻中天麻素的含量均高于药典规定标准。红天麻因生长速度较快,耐旱能力较强,目前在我国栽培面积最广。杂交天麻中天麻素含量最低,部分样品甚至低于药典标准,但在在实际生产中,大多以产量和性状作为选种标准,乌红杂交天麻具有良好的杂种优势,产量最高,性状也较好,正逐渐代替红天麻成为主流栽培品种[96]。

环境因子是影响天麻药材产量和质量的重要因素,在进行综合分析时,应充分考虑起关键性作用的主导因子及其特性,只有这样才能对天麻资源进行准确评价,找出质量调控的关键因子。由变量重要性分析可知,对天麻素影响最重要的气候因子为年降水量。天麻喜细润恶干旱,适宜的降水才能保证天麻健康生长发育,要求年降水量在 1000 mm 左右,同时降雨季节分配也很重要,5 ~9 月生长旺季,需较多降水,到 9 月下旬,雨水过多利于蜜环菌生长,易引起天麻腐烂[97]。其次为年日照时数,但光照并非天麻生长发育必须条件,笔者猜测可能是光照与热量伴随的原因。天麻生理学研究表明,温度决定着天麻和蜜环菌体内酶的活性。温度低积累物质慢,会延长营养生长时间,过高则使生长期缩短,也不利于天麻生长[98]。

相关性分析发现,天麻素与巴利森苷 A、B 均表现出中等程度正相关,与对羟基苯甲醇呈负相关,且巴利森苷 A、B、C 互相呈显著相关性。从化学结构上观察,巴利森苷 A、B、C 均为天麻素和柠檬酸结合而成的酯类。三个化合物的生物合成同时受到两个底物的制约,故表现出很强的相关性;同理,天麻素既是它们的合成底物,自然与天麻素密切相关。而天麻素和对羟基苯甲醇在天麻体内可通过不同的酶促条件进行相互转换,故表现出较强的负相关性。

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(下转第 224 页)