水分胁迫对黄檗幼苗三种生物碱含量的影响

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摘要 黄檗 (Phellodendron amurense)是东北阔叶红松林的重要伴生树种,也是我国名贵中药关黄柏的药源植物,小檗碱 (berberine)、药根碱 (jatrorrhizine)、掌叶防己碱 (palmatine)是研究者通常关注的主要黄檗生物碱。通过池栽法和渗灌控水方式设置了对照、轻度干旱、重度干旱和水涝 (土壤水势分别控制在 -40 ~ -20 KPa、-80 ~ -60 KPa、< -80 KPa 和 -20 ~ 0 KPa)4 种水分处理 ,比较了不同水分状况下黄檗幼苗小檗碱、药根碱及掌叶防己碱含量的变化。对于当年生的黄檗幼苗 ,总体上轻度干旱有利于上述 3 种生物碱的合成与积累 重度干旱处理下幼苗 3 种生物碱的含量与对照差异不大 ,而水涝处理则导致幼苗 3 种生物碱的含量显著降低。同时 ,干旱或水涝处理都导致黄檗幼苗生长受抑 ,幼苗的株高、茎径和生物量都显著低于对照幼苗 ,因此 3 种生物碱的单株产量仍是对照的黄檗幼苗最高。茎外皮是中药黄柏的药用部位 ,也是黄檗幼苗中 3 种生物碱含量最丰的部位 ,实验结果预示短期的轻度干旱处理可以提高茎外皮的小檗碱含量 ,这对以获取生物碱为目的的黄檗幼苗培育可能有参考价值。

关键词 水分胁迫 黄檗 小檗碱 药根碱 掌叶防己碱

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Effects of water stress on berberine, jatrorrhizine and palmatine contents in amur corktree seedlings

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Abstract: Amur corktree (*Phellodendron amurense*) is one of the important accompany species in broad leaved-Korean pine forest, and the pharmaceutical resource plant of famous Chinese traditional medicine cortex phellodendri. Berberine, jatrorrhizine and palmatine are main alkaloids paid high attention by the researchers in amur corktree. In the present study, water stress treatments with four conditions, i. e. mild drought, severe drought, waterlogging and control (soil water potentials were controlled in the ranges of $-40 \sim -20$ KPa, $-80 \sim -60$ KPa, <-80 KPa and $-20 \sim 0$ KPa) were performed using the technique of root-sphere osmotic irrigation in a soil pond. The changes of main medicinal compositions, berberine, jatrorrhizine and palmatine contents under different water conditions were discussed.

Berberine levels in amur corktree seedling differed in various organs. High berberine content was present in stem cortex, 36.6, 1.8 and 142.9 times as much as the peaks in stem xylem, root and leaves, respectively. Berberine

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accumulation induced in amur corktree seedlings after 20 days and significantly increased after 80 days under mild drought treatment. Berberine contents similarly lowered after 60 days and recovered after 80 days under severe drought and waterlogging compared to their respective controls. The variation of berberine content in the root under different conditions was similar to that in stem cortex. Berberine was mainly accumulated in stem cortex and root of seedlings, enhanced under mild drought treatment, whereas decreased under severe drought and waterlogging treatments compared to control (Fig. 1).

Jatrorrhizine content in amur corktree seedlings was much lower than berberine. The stem cortex had relatively more jatrorrhizine, 22.3 and 4.3 times as much as stem xylem and root, whereas no jatrorrhizine was detected in leaves. Jatrorrhizine contents in stem cortex, stem xylem and root under mild drought treatment were higher than that of control. Jatrorrhizine levels in different organs were lower than or close to control under severe drought treatment. The waterlogging treatment lowered Jatrorrhizine levels in amur corktree seedlings. Thus, mild drought may induce the synthesis and accumulation of jatrorrhizine, and waterlogging might reduce jatrorrhizine content in amur corktree seedling (Fig. 2).

Palmatine content in amur corktree seedling was about 10% of berberine. It was abundant in stem cortex, and the maximum value was 20.1, 20.5, or 555 times as much as that in stem xylem, root and leaves respectively. Compared to control, palmatine contents were enhanced under mild drought treatment. No coincident effect on palmatine content was displayed under severe drought during the period of treatment. Under waterlogging, palmatine contents in stem cortex and xylem of the seedling was obviously lower than control. Similar to berberine and jatrorrhizine, mild drought was advantageous to palmatine, and waterlogging would inhibit palmatine in amur corktree seedling (Fig. 3).

The growth of amur corktree seedling was depressed under mild drought and waterlogging treatments. The plant height, stem diameter and biomass were clearly lower than control. Three alkaloids total production in control were the highest, and mainly distributed in stem cortex of seedlings. Our study showed that the mild drought improved the synthesis and accumulation of three alkaloids, but decreased biomass. Therefore, in the process of cultivation, a short time of mild drought treatment would activate alkaloids content, whereas more alkaloid yield would achieve with normal irrigation due to the effective accumulation of plant biomass.

Key Words: water stress; amur corktree (*Phellodendron amurense*); berberine; jatrorrhizine; palmatine

水作为维持植物生存的重要环境因子,可以通过不同的形式、量和持续时间对植物起作用,不同的植物或同一植物的不同发育阶段对水的需求不同^[2-5]。有大量研究工作关注水分胁迫与植物初生代谢的关系,如水分对农作物、经济作物产量和品质的影响^[6-11]。作为植物次生代谢产物的生物碱,是植物在长期进化过程中对环境适应的产物。植物自身的遗传特性控制着生物碱的生物合成,同时一些环境因子(生物的、非生物的)也会诱发、促进或抑制某些生物碱的产生^[12-15]。生物碱能对多种环境胁迫作出响应而在种类与数量上发生变化^[17-20]。探讨植物生物碱的环境调控规律,既可以从次生代谢角度深入分析植物与环境的互作关系,又可为一些产生重要生物碱植物的合理培育提供指导。

黄檗 (Phellodendron amurense)是东北阔叶红松林的重要伴生树种,也是我国名贵中药黄柏的药源植物,其内皮(韧皮部)入药,称为关黄柏。已从黄柏中分离、鉴定出的生物碱有小檗碱 (berberine)、药根碱 (jatrorrhizine)、掌叶防己碱 (棕榈碱,palmatine)、木兰花碱 (magnoflorine)、黄柏碱 (phellodendrine)等,其中小檗碱、药根碱、掌叶防己碱是研究者通常关注的主要生物碱。至今尚缺乏土壤水分对黄檗幼苗主要药用成分影响的基础研究,本项工作通过控制土壤供水,比较了不同水分状况下黄檗幼苗主要药用成分小檗碱、药根碱及掌叶防己碱含量的变化,旨在探讨培育条件对黄檗幼苗药用成分的影响,也为进一步阐明次生代谢与环境的关系提供基础资料。

1 实验方法

1.1 黄檗幼苗培养与水分处理

实验在吉林师范大学温室内进行 (43°9′N ,124°20′E ,海拔 169 m)。 黄檗种子于 0~5℃ 冰箱内沙藏 3 个

月后 温室中播种育苗 其后移栽进行水分胁迫处理。

黄檗幼苗的水分处理采用崔晓阳等的池栽法和渗灌控水方式 [21]。将育好的黄檗幼苗移栽于长 4 m、宽 1.2 m ,深 0.35 m 的处理土壤池 (进一步划分为 4 个小池)中 ,株、行距均为 15 cm ,每池约 200 株黄檗幼苗。水分处理设对照、轻度干旱、重度干旱和水涝 4 种 ,土壤水势分别控制在 -40 ~ -20 KPa、-80 ~ -60 KPa、 < -80 KPa和 -20 ~ 0 KPa。 水涝处理池土壤下铺有塑料布 ,保持土壤表层处于轻微积水状态。重度干旱处理实际上已超出张力计量程范围 水分控制以中午时黄檗幼苗发生暂时性轻度萎蔫为准。每处理重复 5 次 (5 小池) ,按随机区组排列。控水 120 d。

1.2 取样与生物碱含量测定

2004 年 6 月 10 日开始控水处理至 10 月 8 日 ,每隔 20 d 于 8 $00 \sim 9$ 00 时取样 ,共取样 7 次。各处理分别在每个重复池内生长正常的黄檗幼苗中随机抽取 1 株 ,测量幼苗的株高、茎径 (基部),进一步分为根、茎木质部、茎外皮和叶片 80% 烘干至恒重后称重、测定生物碱含量 (重复为 5)。

小檗碱、药根碱和掌叶防己碱含量按秦彦杰等 $^{[2]}$ 方法使用美国 Waters 高效液相色谱 (2996 自动进样器、2487 紫外检测器)测定。样品以 60% 乙醇超声提取 60 min ,色谱柱为日本 KYA HIQ sil C18 柱 (250 mm × 4.6 mm $_5$ μ m) ,流动相为乙腈/水溶液 (1:1 ,1000 ml 溶液中含磷酸二氢钾 3.4 g 及十二烷基磺酸钠 1.7 g) ,流速 1.0 ml/min ,进样量 10 μ l 检测波长 345 nm。小檗碱和药根碱标准品购于中国药品生物制品检定所 ,掌叶防己碱标准品购于美国 Adrich 公司。

2 结果与分析

2.1 水分胁迫对黄檗幼苗小檗碱含量的影响

从小檗碱含量最高的茎外皮看 轻度干旱环境下从处理的第 20 天起小檗碱含量持续最高到最后 ,并从处理 80 d 起与其他处理有显著差异 (p < 0.01)。 重度干旱和水涝处理的黄檗幼苗小檗碱含量相近 ,处理的第 60 天起表现为低于对照 ,但到处理末期 (10 月 8 日)又接近对照水平。根的情况与茎外皮类似 ,也是轻度干旱的小檗碱含量最高 ,而重度干旱和水涝处理的接近或低于对照的小檗碱含量 (图 1)。

2.2 水分胁迫对黄檗幼苗药根碱含量的影响

黄檗幼苗的药根碱含量远低于小檗碱含量,大约为小檗碱含量的3%。不同部位的药根碱含量也是茎外皮最高,最高值分别为茎木质部和根的22.3倍和4.3倍,而叶片中没有测出药根碱。茎外皮的药根碱含量随着发育进程而迅速增加,而根的药根碱含量在发育进程中呈现平缓的单峰曲线,处理80d时为最高值(图2)。

轻度干旱处理的黄檗幼苗,茎外皮的药根碱含量在处理 60~d 以后有高于对照的趋势,但差异并不显著 (p>0.05),而重度干旱处理的药根碱含量低于或接近对照,差异也不显著 (p>0.05)。 水涝处理的从处理 60~d 起药根碱含量显著低于对照 (p<0.01)。 根的药根碱含量在轻度干旱处理 40~d 时显著高于对照,而其他生育时期与对照接近。重度干旱处理的黄檗幼苗根的药根碱含量在处理后期有低于对照的趋势,但差异不显著 (p>0.05),而水涝处理的在后期则明显低于对照 (p<0.01) (图 2)。

2.3 水分胁迫对黄檗幼苗掌叶防己碱含量的影响

黄檗幼苗的掌叶防己碱含量约为小檗碱含量的 10%。不同部位的掌叶防己碱含量也是茎外皮最高,最

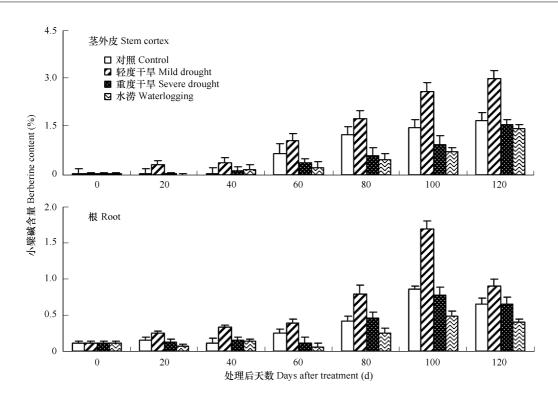


图 1 水分胁迫下黄檗幼苗小檗碱含量的变化

Fig. 1 Changes of berberine content of amur corktree seedlings under water stress

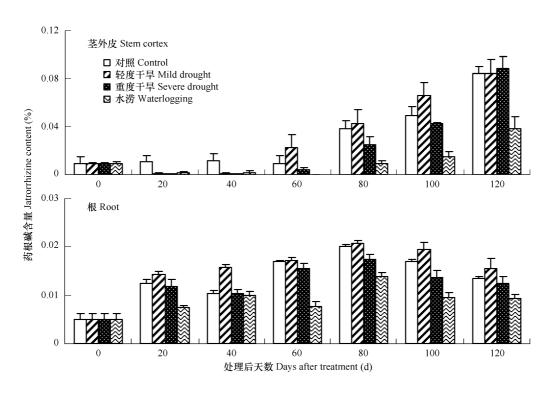


图 2 水分胁迫下黄檗幼苗药根碱含量的变化

Fig. 2 Changes of jatrorrhizine content of amur corktree seedlings under water stress

高值分别为茎木质部和根的 20.1 倍和 20.5 倍 ,而叶片的掌叶防己碱含量最高值仅为茎外皮的 0.18%。茎外皮和根的掌叶防己碱含量基本上是随着发育进程而增加 ,但根在处理后期 (处理 100 d 至 120 d)掌叶防己碱

含量变动不大(图3)。

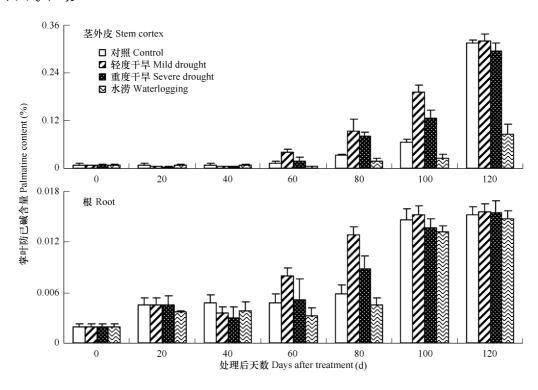


图 3 水分胁迫下黄檗幼苗掌叶防己碱含量的变化

Fig. 3 Changes of palmatine content of amur corktree seedlings under water stress

轻度干旱处理的在 $60 \sim 100~\mathrm{d}$ 、重度干旱处理的在 $80 \sim 100~\mathrm{d}$,黄檗幼苗茎外皮的掌叶防己碱含量高于对照 (p < 0.01),而在其他时期则与对照接近。水涝处理的黄檗幼苗,茎外皮的掌叶防己碱含量从处理 $80~\mathrm{d}$ 起显著低于对照和干旱处理 (p < 0.01)。黄檗幼苗根的掌叶防己碱含量只有轻度干旱处理 $60~\mathrm{d}$ 和 $80~\mathrm{d}$ 的高于对照 (p < 0.01),其余时期各处理间差异不明显 (p > 0.05) (图 3)。

在黄檗幼苗临近生育期末的生长后期,干旱处理呈现有利于茎外皮掌叶防己碱合成与积累的趋势,而水 涝处理则不利(图3)。

2.4 水分胁迫对黄檗幼苗生物量及3种生物碱单株产量的影响

表 1 是最后一次取样 (10 月 8 日 ,处理 120 d)测定的结果。可以看出 ,土壤水分对黄檗幼苗生长有明显的影响 ,无论是干旱处理还是水涝处理 ,都严重抑制了黄檗幼苗的生长 ,幼苗的株高、茎径和生物量都显著低于对照幼苗。

表 1 黄檗幼苗生物量及生物碱单株产量

Table 1	Biomass	and	alkaloid	production	of	amur	corktree	seedlings
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项目	株高	茎径 Stem	生物量	小檗碱	药根碱	 掌叶防已碱
Intem	Height (cm)	diameter (mm)	Biomass (g/plant)	Berberine (mg/plant)	Palmatine (mg/plant)	Jatrorrhizine (mg/plant)
对照 Control	53. 6a	7. 7a	18. 96a	76. 34a	2. 22a	5. 85a
轻度干旱 Mild drought	35. 5b	5. 5b	8. 87b	69. 76a	1. 56b	4. 16b
重度干旱 Severe drought	29. 0c	4.8c	4. 90c	24. 00b	0.80c	2. 10c
水涝 Waterlogging	18. 9d	4.5c	2. 61 d	11. 10c	0. 28d	0. 53 d

同列中不同字母表示差异显著 Different letters in same columns denote the significant differences (p < 0.05)

尽管轻度干旱处理有提高黄檗幼苗生物碱含量 (尤其是茎外皮的小檗碱和掌叶防己碱含量)的趋势 ,但

由于处理幼苗的生物量尚不及对照幼苗的一半。因此3种生物碱的单株产量仍是对照幼苗最高,重度干旱和水涝处理的幼苗则更是低于对照(表1)。

3 结语与讨论

从前述实验结果可以看出,对于当年生的黄檗幼苗,总体上轻度干旱 (土壤水势 $-80 \sim -60$ KPa)有利于主要药用成分小檗碱、药根碱和掌叶防己碱在茎外皮的合成与积累,重度干旱 (土壤水势 <-80 KPa)处理下幼苗 3 种生物碱的含量与对照 (土壤水势 $-40 \sim -20$ KPa)差异不大,而水涝处理 (土壤水势 $-20 \sim 0$ KPa)基本上是导致幼苗 3 种生物碱的含量降低 (特别是在茎外皮)。同时,干旱或水涝处理都导致黄檗幼苗生长受抑,幼苗的株高、茎径和生物量都显著低于对照幼苗。因此,从 3 种生物碱的单株产量看,仍是对照的黄檗幼苗最高 (表 1)。

一般认为 植物的次生代谢及其产物是植物在长期进化过程中为响应和适应各种逆境而形成的 ,次生代谢产物的形成与环境因子 (生物的和非生物的)有着密切的联系 ,生物碱类次生代谢产物通常在植物抵御昆虫和食草动物的化学防御过程中扮演着重要的角色 [23]。轻度干旱处理导致黄檗幼苗茎外皮的生物碱含量增加 ,意味着生物碱代谢与非生物环境之间也可能有一定的联系 ,但这种联系可能并不像与生物环境之间的关系 (化学防御)那样直接 ,这需要深入的机理研究来阐明。另一方面 ,许多研究者认为次生代谢产物的产生是植物以牺牲生长为代价的 ,在环境胁迫程度足以影响到植物的生命时 植物就只能以生长为主要目的而相应地减少次生代谢产物的生产 [19]。如果假设生物碱代谢与非生物环境之间确有一定的联系 ,那就可以解释重度干旱和水涝处理时黄檗幼苗生物碱含量的降低 ,因为重度干旱和水涝处理都严重影响了黄檗幼苗的正常生长。

茎外皮是中药黄柏的药用部位,也是黄檗幼苗中3种生物碱含量最高的部位。3种生物碱在茎外皮中均有一个随发育进程而含量逐步增加的过程。尽管水分处理效果在后期(处理 100 d 时)比较明显,但在处理 60 d 时 轻度干旱对3种生物碱合成与积累的促进作用已经显现出来(图1、图2、图3),这就意味着短期的轻度干旱处理也可能提高黄檗幼苗的生物碱含量。因此,在以获取生物碱为目的的黄檗幼苗培育过程中,可以考虑先给以黄檗幼苗正常的水分供应以获得最大的生物产量,然后在适当的时期施以轻度干旱处理以提高生物碱含量,这样就有可能获得超过正常培育的生物碱单株产量。当然,这只是一种推测,需要进一步的设计实验观察来验证。

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