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杨树耐旱性的生理生化基础

Physiological and Biochemical Mechanisms of Drought Resistance in *Populus*

陈少良

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前　言

我国广大的三北地区，限制林业发展的主要因素是干旱，雨量不足而且集中在7、8月，这是限制人工林生长和影响造林成活率的主要因子。在树木耐旱性方面的研究工作已有很多，但其中很少涉及我国北方地区广泛栽培的树种——杨树的耐旱性问题，而实际上不同杨树种类之间的耐旱性相差很大。研究不同杨树的耐旱性差异，不但有利于干旱地区更好地利用较耐旱的杨树、提高人工林的郁闭速度、尽早发挥生态环境效益，而且还可以增加人工林的材积及生物量以满足当地人民生产与生活水平不断提高的需要。从树木耐旱性的理论研究上讲，对比研究属内耐旱性相差较大的种的生理生化反应，有利于寻找更能反映耐旱性本质的指标，可加深我们对树木耐旱机制的认识，为树木抗旱性遗传改良铺平道路。这就是德国—以色列农业研究协定(GIARA)研究项目“提高中国速生树种在逆境下生产力”(1992-9—1995-12)的立项意图。本文所研究的杨树耐旱性差异及其生理生化基础，是该项目的一个组成部分。

摘　　要

作为“提高中国速生树种在逆境下生产力”（德国—以色列农业研究协定，GIARA）科研项目的一部分，本文对不同种类杨树耐旱性的生理生化基础进行了研究。研究材料涉及：I-214杨（*Populus* ×*euramericana* cv. I-214），健杨（*Populus* ×*euramericana* Guinir cv. robusta），小叶杨与钻天杨的杂交种——群众杨（*P. popularis* 35-44），苦杨与钻天杨的杂交种——中东杨（*P. berolinensis*）等4种杨树。在控制土壤湿度条件下，比较了一年生插条苗受旱后的生长、光合作用、水分状况等方面的变化；分析了限制杨树光合作用的气孔因素和非气孔因素；研究了土壤水分亏缺和ABA对多胺、乙烯生物合成的影响及其与叶片衰老脱落的关系。首次从根冠通讯的角度揭示了不同耐旱性杨树适应水分逆境差异的机制，提出并初步证明了根冠通讯复合化学信号的假说。另外，还在分子水平上初步研究了不同耐旱性杨树逆境胁迫蛋白的差异。具体研究结果简述如下：

1. 在土壤水分充足供应的条件下（90%田间持水量，FFC，土壤水势为-0.082 MPa）大气水分亏缺显著抑制了4种杨树的净光合速率（Pn）。但比较而言，中东杨忍耐大气干旱的能力最强，在大气干旱条件下Pn和水分利用效率（WUE）最高，原因在于气孔对低湿空气反应的不灵敏性。

2. 在高土壤湿度下（70%～100% FFC，土壤水势为-0.056～-0.122 MPa），I-214杨的Pn和生长速率最高，健杨次之。但土壤干旱对这两种杨树影响最大：当土壤湿度降至40% FFC（土壤水势为-0.824 MPa）时，I-214杨和健杨的Pn即有显著降低，严重干旱（30% FFC，土壤水势为-2.108 MPa）时，Pn受到严重抑制；并使叶面积生长下降51%和37%，高生长基本或接近停顿，自下

而上落叶达全株总量的 1/4 ~ 1/3。群众杨和中东杨在严重干旱时 Pn 才显著下降,Pn 在受旱后第 8 天有明显回升,分别恢复至对照水平的 88%(群众杨)和 78%(中东杨),并且未导致落叶。总之,在 30% FFC 条件下,群众杨在 4 种杨树中生产力水平最高,而 I - 214 杨则最低。

3. 研究干旱胁迫(30% FFC)对 4 种杨树受旱苗木光合作用和叶绿素 a 活体荧光的影响,结果显示,恒定荧光未受影响,而最大荧光有所提高,并导致可变荧光与恒定荧光的比值较对照提高 20% ~ 50%。鉴于光抑制是以最大荧光或可变荧光的下降为特征的,因此得出 PSⅡ光反应中心未受伤害的结论。试验结果显示,不耐旱杨树(I - 214 杨和健杨)受旱后 Pn 剧烈下降的原因,主要是非气孔因素影响,即 RuBP 羧化酶活性的降低。而对于耐旱杨树,受旱后 Pn 下降幅度较小,气孔因素和非气孔因素的限制作用大体相当。

4. 监测土壤干旱(30% FFC)期间受旱植株叶片含水量、凌晨水势、中午水势的结果显示,不耐旱杨树(I - 214 杨和健杨)叶片水分亏缺程度高于耐旱的群众杨和中东杨。其中,群众杨叶片持水力以及苗木维持水分平衡的能力最强,细胞壁弹性最大,即其维持膨压的能力最强。干旱胁迫使群众杨叶片脯氨酸水平增高 40%,表明在干旱初始阶段有较明显的渗透调节发生。中东杨的细胞壁弹性仅次于群众杨,土壤干旱使束缚水含量提高 18%,并且膨压最大时的渗透势和膨压为零时的渗透势分别降低 0.08 MPa 和 0.07 MPa,因此,这种杨树具有较强的渗透调节能力。不耐旱的 I - 214 杨和健杨的细胞壁弹性最小,但干旱可诱导其提高。这两种杨树受旱伊始,即发生严重的水分亏缺(凌晨水势比对照降低 0.4 ~ 0.5 MPa),脱落部分叶片后,水分状况才得以恢复。

5. 干旱胁迫对 I - 214 杨多胺和乙烯生物合成途径的影响,可分为两个阶段:

第一阶段：在中度干旱（40% FFC）处理初期，多胺生物合成受到影响，表现在腐胺（Put）和精脒（Spd）水平的明显下降和精胺（Spm）的消失。由于 Spd 和 Spm 合成受阻，使 SAM（*S* - 腺苷蛋氨酸）累积而使乙烯生物合成途径加强，乙烯释放量显著提高，但 ACC/MACC（丙二酰 ACC）比值始终在 1 以下，即 ACC 合成酶活性提高的同时，ACC 丙二酰转移酶活性也有所提高。

第二阶段：严重干旱（30% FFC）时，SAM 合成也受到抑制，但 ACC/MACC 却大幅度上升至 1 以上。多胺生物合成受到进一步削弱，Spd 消失，此时发生老叶自下而上的脱落。木质部导入的 ABA（10 ml，200 μmol/L）能诱导 I - 214 杨叶片的多胺和乙烯生物合成途径发生类似于干旱胁迫下的变化和落叶。严重干旱（30% FFC）条件下，群众杨不落叶，多胺生物合成受影响较小，Spd 不消失；ACC/MACC 始终保持在 1 以下，并且干旱植株乙烯释放速率的变化幅度小，乙烯的生物合成处于相对不活跃的状态。

根据在严重干旱条件下落叶的 I - 214 杨和不落叶的群众杨的多胺及乙烯生物合成方面所表现出的差异，初步结论是，当干旱胁迫使多胺生物合成途径削弱到组织内的精脒消失、并且乙烯合成相对增强到 ACC/MACC 的比值超过 1 时，则落叶发生。

6. 通过木质部导入脱落酸（ABA）和细胞分裂素（Cyt）以模拟干旱胁迫条件下蒸腾流中这两类激素浓度的变化，研究 ABA 和 Cyt 对气孔行为的调控。结果显示，随蒸腾流导入的 ABA 可以诱导气孔关闭，而与 ABA 共导入或分别导入的 Cyt 可以降低气孔对 ABA 的敏感性。过去的研究已经证明，水分胁迫条件下根系合成 ABA 能力提高的同时，Cyt 的合成及运输受阻。所以在根冠通讯中，是根源 ABA 和 Cyt 共同调控气孔运动。因此根冠通讯的化学信号是一复合信号，即 ABA 与 Cyt 比例的高低决定着气孔行为。通过比较玉米素、激动素和 BA（6 - 苄基腺嘌呤）等不同种类细胞分裂素与 ABA 的相互作用，结果表明激动素与植物内源激素玉米素的作用相同，也能拮抗 ABA 对气孔运动的影响，而 BA 的作用

相反。

7. 群众杨在受旱第1天，根系中ABA的水平即达到峰值，为对照处理的4.0倍，木质部汁液中ABA的浓度[ABA]也随之达到对照水平的4.5倍。由于内源ABA水平的提高是在植物体水分状况没有发生明显亏缺的情况下发生的，因此这种根冠通讯属前胁迫反应。I－214杨内源ABA水平的提高发生在植株水分状况出现显著下降(受旱植株凌晨水势低于对照处理0.50 MPa)时，并随胁迫时间而持续上升，第3天才达到峰值，分别为对照水平为7.0倍(根系)和10.0倍(木质部[ABA])。因此这种根冠通讯属于胁迫反应。群众杨适应干旱的前胁迫反应机制表明根系在干旱初始阶段即能感知土壤水分亏缺并合成化学信号ABA上运至叶片，实现对气孔行为的调控，可能与其他适应性调节(如渗透调节)一起建立新的水分平衡。随后ABA水平逐渐降低并恢复至对照水平，叶片的气孔导度和气体交换也随之明显回升。I－214杨对干旱胁迫反应机制表明根系是在植株发生显著水分亏缺时才大幅度增加ABA的合成，到第3天达到引起落叶的水平，因此主要是通过部分叶片脱落来重建水分平衡的。

8. 在地上部对ABA应答方面，群众杨气孔对ABA反应最敏感，I－214杨次之，中东杨的敏感性最低。在生长反应方面，木质部导入的ABA(10 ml，200 μmol/L)显著抑制了I－214杨高及叶面积生长，而群众杨则基本未受影响。ABA所诱导的I－214杨的落叶量为18%(70% FFC＋ABA)和37%(40% FFC＋ABA)，分别高于群众杨的落叶量：10%(70% FFC＋ABA)和25%(40% FFC＋ABA)。因此，群众杨气孔对ABA的反应阈值低，而生长和落叶则对ABA的反应阈值高。I－214杨的情况刚好相反。

9. 在分子水平上初步研究了不同耐旱性杨树逆境胁迫蛋白的差异。热激处理能诱导群众杨叶片合成特异性蛋白质，相对分子质量分别为70 700、48 400和12 900。水分胁迫处理也能诱导70 700多肽的出现。而相应的处理并未诱导I－214杨叶片产生

特异性蛋白质。因此,新蛋白质的诱导产生对于耐旱的群众杨适应干旱逆境以及与之相关的高温逆境可能是很重要的。PEG处理能诱导根系合成多种新的蛋白质,并且I-214杨、中东杨和群众杨的情况近似。但耐旱的群众杨和中东杨的PEG诱导蛋白具有一定的热稳定性,而I-214杨的诱导蛋白则是热不稳定性的。

关键词:杨树,抗旱性,根冠通讯,ABA,细胞分裂素

ABSTRACT

As one part of GIARA (German-Israeli Agricultural Research Agreement) project "Improving productivity of fast-growing trees under adverse conditions in China", this paper aimed at elucidating the genotypic difference of *Populus* in physiological and biochemical mechanisms of drought resistance. Experiments were carried out in pots with 1-year-old rooted cuttings of 4 poplar genotypes: *Populus* × *euramericana* cv. I-214 (Italica); *Populus* × *euramericana* Guinir cv. robusta (Robusta); *P. berolinensis* (Berolinensis), the hybrid of *P. laulifolia* × *P. nigra* var. italica; *P. popularis* 35-44 (Popularis), the hybrid of *P. simonii* × (*P. pyramidalis* + *Salix matsudana*). The effects of water stress on growth, photosynthesis and water status were investigated, and stomatal, non-stomatal limitation of CO_2 assimilation of the 4 genotypes were analyzed. The influence of water stress and external ABA application on polyamine, ethylene biosynthesis and the relevance to leaf abscission were examined. Genotype-specific differences in mechanisms of root-shoot communication of poplar in response to drought were elucidated for the first time, and the concept of a compound root-originated signal —— ABA/cytokinins was put forward and preliminarily conformed. Furthermore, the difference between drought-sensitive and drought-tolerant genotypes in stress-responsive polypeptides were compared and it was considered as molecular mechanisms involved in drought tolerance. The main results of the paper are detailed as follows:

1. Under optimal moisture of 90% FFC (full field capacity, soil water potential -0.082 MPa), net photosynthetic rates (Pn) of 4 ex-

perimented genotypes were limited by air drought. Comparatively, Berolinensis was the most tolerant to air drought because its stomata were insensitive to low air humidity, allowing to keep a relatively high *stomatal conductance for gas exchange(Pn, TRN) and water use efficiency.*

2. Under high soil moisture of 70% ~ 100% FFC (soil water potential −0.056 ~ −0.122 MPa), Italica kept the highest Pn and growth rate and Robusta was next. However, Italica and Robusta were very responsive to soil drought and significant reductions of Pn appeared when soil moisture was lowered to 40% FFC. After subjected to severe soil drought (30% FFC, soil water potential −2.108 MPa), Pn of the two qenotypes was dramatically suppressed and droughted plants shed about one quarter to one third of their leaves. Growth of expanding leaves was decreased by 51% (Italica) and 37% (Robusta), respectively, whereas a less effect on leaf growth was found in Berolinesis and Popularis, and they had no leaf abscission at all during the period of water stress. Although the severe soil drought significantly decreased Pn of Berolinensis and Popularis as well in the *first several days, an obvious recovery of Pn displayed in the 8th day of* exposure to water shortage. In conclusion, Popularis kept the highest productivity under soil moisture of 30% FFC among the 4 poplar genotypes and Italica was the lowest.

3. The effects of water stress on photosynthesis and chlorophyll a (Chl a) fluorescence were investigated in the 4 poplar genotypes. Constant fluorescence was not affected by increasing water stress while the ratio of variable to constant fluorescence increased by 20% ~ 50%, *implying there was no injury to photosystem II reaction center,* as photoinhibition is characterized by a progressive decline in maximal or variable fluorescence. Net photosynthetic rates of Italica and Ro-

busta, which are more sensitive to water stress, were drastically reduced due to non-stomatal factors; their activity of ribulose bisphosphate carboxylase-oxygenase (Rubisco) was also severely inhibited. For Berolinensis and Popularis, the two more drought tolerant poplar genotypes, stomatal and non-stomatal components played equally important roles, and only a moderate or a light decrease of net photosynthetic rate was detected in response to water stress.

4. During the period of water stress, the changes of leaf water content, predawn and midday water potential indicated that water deficit was developed higher in droughted plants of drought-sensitive poplar genotype than that of drought-tolerant ones. And Popularis showed the great capacity in keeping water balance among the 4 experimented genotypes. Furthermore, the highest cell wall elasticity of Popularis indicates its stressed-plants have the advantage of maintaining turgor as water potential declines. After subjected to severe soil drought (30% FFC), leaf proline content of Popularis increased by 40%, implying osmotic adjustment occurred after the onset of water stress. For Berolinensis, its cell wall elasticity was lower than that of Popularis, however, its bound water (Va) increased by 18% after drought treatment and Ψ_{π}^{100} (osmotic potential at saturation), Ψ_{π}^{0} (osmotic potential at turgor loss) decreased by 0.08 and 0.07 MPa, respectively. Therefore, Berolinensis may maintain turgor mainly through osmotic adjustment during the period of water stress.

Compared with the other two drought-tolerant genotypes, cell wall elasticity of Italica and Robusta was low, however, it can be improved certain a extent by water stress. Plant water status of Italica and Robusta was greatly affected during the onset of soil drying (predawn leaf water potential decreased by 0.4 ~ 0.5 MPa) and it recovered after leaf abscission.

5. There were two stages in the changes of polyamine and ethylene biosynthesis after drought treatment:

The first stage: In this stage, significant decrease of putrescine (Put) and spermidine (Spd), and disappearance of spermine (Spm) after mild water stress (40% FFC) indicated polyamine biosynthesis was obviously inhibited, resulting in a SAM (*S*-adenosylmethionine) accumulation and the biosynthesis and release rate of ethylene increased subsequently. However, the ratio of ACC (1-aminocyclopropane-1-carboxylic acid) and MACC (1-(malonyamino) cyclopropane-1-carboxylic acid) was lower than 1 implying the malonyl transferase activity increased simultaneously with that of ACC synthase.

The second stage: After subjected to severe drought (30% FFC), SAM biosynthesis was inhibited and the ratio of ACC/MACC exceeded 1. In the meantime polyamine biosynthesis greatly decreased and no Spd was detected. Leaf abscission occurred at this stage. Xylem-fed ABA solution (10 ml, 200 μmol/L) caused similar changes of polyamine and ethylene as that of water stress. In comparison with Italica, polyamine biosynthesis in Popularis leaves was less affected and Spd was detected after plants subjected to severe drought, and no leaf abscission was observed during the period of water stress. Ethylene release rate was less affected by drought and ACC/MACC never went beyond 1. Therefore, ethylene biosynthesis in Popularis leaves was not significantly activated under water stress.

Based on the genotypic differences in leaf abscission and in polyamine and ethylene biosynthesis, It is preliminarily concluded as that when leaf Spd is undetectable and ACC/MACC exceeds 1, leaf abscission ultimately occurs.

6. The role of exogenous ABA and cytokinins in stomatal control were examined by xylem feeding to poplar genotypes. Xylem-fed ABA

readily induced stomatal closure, however its strong inhibitory effect could be distinctly overcome by cytokinins which were supplied either separately, or together with ABA via the transpiration stream. Since endogenous ABA is known to increase while cytokinin biosynthesis in roots and its transports were inhibited by drought, the concept of a compound root-originated signal of ABA/cytokinins was put forward and discussed. It was assumed that the ratio of ABA and cytokinins in transpiration stream controlled stomatal behaviour under water stress. In addition, the interactions between ABA and different forms of cytokinins including zeatin, kinetin and 6-benzyladenine (BA) were also investigated. Results showed that BA, an artificial cytokinin enhanced the inhibitory effect of ABA instead of overcoming its influence which was contrary to zeatin and kinetin.

7. On the first day of exposure to water stress (30% FFC), ABA content in roots of Popularis reached to its peaking level, which was 4 times as that of control plants, and at the same time a 4.5-fold increase of xylem ABA concentration [ABA] was observed in droughted-plants. As the rise of endogenous ABA in Popularis occurred before the decline of plant water status, therefore it was defined as a pre-stress response in root-shoot communications. By contrast, the rise of endogenous ABA occurred after the decline of plant water status in Italica (a 0.50 MPa decrease of predawn leaf water potential was observed in stressed-plants) and it continued to increase and reached to its peaking level on the third day after drought treatment, which was 7.0-fold and 10.0-fold as that of control in roots and xylem transpiration stream, respectively. Therefore, the changes of ABA level in droughted-plants of Italica was typically a stress response in root-shoot communication. Pre-stress response of Popularis in response to drought implies that the roots can sense the soil drying during the on-

set of water stress and synthesize the drying signal—ABA to communicate with shoots. Therefore stomatal movements can be adjusted and together with other additional adjustments (e. g. osmotic adjustment) are considered involved in creating a new water balance under soil water adverse condition. Afterwards, stomatal conductance recovered gradually coincident with the gradual decline of xylem [ABA]. Stress response of Italica in response to drought shows that the roots synthesize the drying signal — ABA to communicate with shoots only after significant deficit of plant water status. And ABA continues to rise up to the level that can induce leaf abscission. Therefore, stressed-plants of Italica create a new water balance mainly by decreasing evaporative surface area.

8. Genotype differences were also observed in sensitivity of shoots responding to ABA. Stomatal movement of Popularis was the most sensitive to the drying signal, while Italica was next and Berolinensis was not sensitive. Shoot growth of Italica were sensitive to water stress, ABA application (10 ml, 200 μmol/L) caused greater declines in stem height and leaf area growth compared with Popularis. Furthermore, ABA application induced more leaf loss in Italica. Therefore, the threshold of ABA affecting growth and leaf abscission is higher in Popularis than that in Italica.

9. Genotype difference in stress-responsive polypeptides were compared. Heat shock treatment induced synthesis of 70 700, 48 400 and 12 900 polypeptides in Popularis and water stress induced synthesis of 70 700 polypeptide. However, no stress-responsive protein was observed in Italica. Therefore, newly-synthesized proteins of Popularis were considered as an important mechanism in its adaptation to adverse conditions of drought and high temperature. PEG-responsive polypeptides were similar in roots of Italica, Berolinensis and Popu-

laris. However, PEG-induced proteins of drought-tolerant genotype — Popularis and Berolinensis were heat stable, but not for Italica.

Key words: *Populus*, drought tolerance, root-shoot communications, ABA, cytokinins

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