Root shape adaptation to mechanical stress derived from unidirectional vibrations in *Populus nigra*

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Abstract  While it is known that plant roots can change their shapes to the stress direction, it remains unclear if the root orientation can change as a means for mechanical reinforcement. When stress in form of a unidirectional vibration is applied to cuttings of *Populus nigra* for 5 min a day over a period of 20 days, the root system architecture changes. The contribution of roots with a diameter larger than 0.04 cm increases, while the allocation to roots smaller than 0.03 cm decreases. In addition to the root diameter allocation, the root orientation in the stem proximity was analyzed by appearance and with a nematic tensor analysis in an attempt to calculate the average root orientation. The significant different allocation to roots with a larger diameter, and the tendency of roots to align in the vicinity of the stress axis (not significantly different), are indicating a mechanical reinforcement to cope with the received strain. This work indicates an adaptive root system architecture and a possible adaptive root orientation for mechanical reinforcement.

Key words:  mechanical stress, plant biomechanics, root orientation, shape adaptation.

Introduction

Plants are constantly exposed to mechanical forces derived from a static load by its own weight and from dynamic external forces such as winds (Moulié et al. 2011; Niklas 1992; Niklas and Spatz 2000). In addition, plants undergo mechanical stresses that come from internal origins such as tissue development (Hamant 2013). These external and internal mechanical stresses can be reformulated by the term mechanical instability: slow instability by increasing own weight and height, and fast instability by drastic environmental change induced by winds (Gardiner et al. 2010, 2016). To resist these mechanical instabilities, it is reasonable to think that the plants potentially have an ability to change its form to adapt to the mechanical stresses, termed thigmomorphogenesis.

Especially for the plant roots, as an intuitive example, they distribute to be wide spread in order to anchor to the soil and stabilize the stem. Moreover, the analysis of a tree root system in response to directional wind showed that roots aligned in the force direction undergo mainly bending and compression, depending if they are on the upwind or downwind side respectively (Coutts 1983; Ennos 2000). Interestingly, the mechanical stress received by the shoot is transmitted to the roots, altering the root system (Stubbs et al. 2019). Such morphological changes upon mechanical stress were detected in various plant species, ranging from grasses over shrubs to trees (Jaffe and Forbes 1993). However, there is little information about adaptive growth processes of plant roots and a preferable root orientation upon mechanical stress.

In this study, we revealed the adaptation of *P. nigra* hardwood cutting roots to unidirectional vibrations by assessing the root distribution quantitatively. The interplay of the root distribution combined with a root structure analysis is discussed.

Materials and methods

Plant growth conditions

Cuttings with 20 cm length and a diameter between 2–5 mm were taken from *P. nigra* var. *italica* branches. The lower half was defoliated, and the lower end dipped in the rooting hormone mix Luton from Sumitomo Chemical Co. Ltd. Root growth was initiated for 10 days in a shaded, high-humidity chamber. After root growth initiation, plants were transferred to 20 cm diameter ceramic pots with a mixture of 9/10

Abbreviations: RSA, Root System Architecture; NTA, nematic tensor analysis.

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akadama earth, 1/10 organic fertilizer from leaves, and 3 g per pot of nitrogen/phosphorus/potassium (N/P/K) fertilizer. All experiments were conducted between July and December in a greenhouse at 28°C with natural sunlight supplemented by artificial light between 5 a.m. to 7 p.m. The plants were grown further 10 days before the stress induction experiment start.

**Mechanical stress infliction**

To inflict mechanical stress in a reproducible manner, a reciprocal shaker model NR-30 from Taitec Cooperation was used. The acceleration at different rotations per minute was measured with the Science Journal application (Google 2020) which uses a mobile phone integrated accelerometer. The unidirectional vibrations at 61 rpm inflicts a peak linear acceleration of 1.3 m/s².

All plants of the stress group were treated 5 min a day for 20 consecutive days, while the control group was not. A label indicating the shaking orientation was used to inflict the stress always in the same axis, and to mark the axis for later distribution analysis.

**Physiological analysis**

The general influence on the growth was determined on the 20th day by comparing the shoot and root fresh weight ratio, after the plant was freed from soil and the root was washed. Pictures with a digital camera were taken for growth comparison. To analyze the root distribution, the shoot was cut 5 cm above the root initiation area. The root was placed on a reversed 200 ml plastic beaker to take a picture from above. The placement itself did not influence the root orientation, since the root protruding from the stem are relative stiff.

**Quantitative analysis of root distribution**

A quantitative analysis of the root distribution was performed based on a nematic tensor analysis (NTA) with an incorporation of error estimations, as previously developed for the estimation of cortical microtubule orientation (Tsugawa et al. 2016). In short, binarized pictures of the root orientation, with the previous shaking direction horizontally aligned, and with a known scale were used. With the shoot position forming the center location, anisotropy vectors were aligned to the binary picture in a range from 0 to π/2, corresponding to the respective root orientation. The frequency of the anisotropy vectors was used to calculate the probability of the root orientation for single roots. With individual roots grouped based on their treatment, the average root orientation in the control and the stress group were calculated independently.

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![Figure](image_url)

**Figure 1.** Root and shoot growth under repeated unidirectional vibrations. Representative pictures of control (A, C) and stressed (B, D) shoots and roots after 20 days. Scale bar=10 cm. The root and shoot freshweight (E) and the shoot/root freshweight ratio (F) are shown in boxplots. Black column=control group, gray=stress group. n=5-7. One out of three experimental setups is shown.
Root system diameter distribution

To analyze the root system architecture, the roots were cut and scanned at 600 dpi resolution in water filled plastic boxes placed on an Epson GT-X970 flatbed scanner. The Smart Root plugin (Lobet et al. 2011) for Fiji (Schindelin et al. 2012) was used to determine the length and diameter of individual roots. Based on the diameter, the roots were grouped into lateral roots with <0.03 cm, lateral roots and small adventitious roots with >0.03 cm and <0.04 cm and large adventitious roots with >0.04 cm in diameter. The stress and control group were compared based on their root diameter group contribution to the total root length, surface and volume, all presented in %.

Results

Growth analysis after repeated unidirectional vibrations

To analyze the effects of repeated unidirectional vibrations on rooted P. nigra cuttings, the shoot and root growth was compared after 20 days between the control group and the stress group. While the shoot growth showed no obvious differences (Figure 1A, B), the roots of the stressed samples looked more dispersed compared to the control samples (Figure 1C, D). However, although the shoot and root freshweight and the shoot/root ratio showed a tendency to be increased by the stress, no significant differences were detected (Figure 1E, F). This indicates that the shoot/root allocation was not influenced by repeated unidirectional vibrations.

Quantifying the root distribution

While no weight allocation was observed, the root pictures in Figure 1 indicated a difference in the root structure. To analyze the aforementioned dispersed look of the stressed group, the attention was focused to the roots originating from the shoot base. Since the roots protruding from the shoot base are the oldest and thickest, they are relatively stiff and will hold therefore their form when placed on a beaker, which allows for the distribution analysis. For this purpose, the shoots were removed and root distribution of the control group (Figure 2A) and stressed group (Figure 2B) was observed from above. The roots of the control group tend to be not so dispersed (Figure 1C) and no clear orientation preference can be seen (Figure 2A). On the other hand,
the roots of the stressed group seem to have a different distribution compared to the control group (Figure 2B).

To have a more objective view on the root distribution, the setup was quantitatively analyzed. A representative graphical display for this procedure is presented in Figure 3C (see also Material and methods). In Figure 3C, the four roots on the left represent the control group and four roots on the right represent the stress group. With three panels of each line, it shows the binary picture of the root orientation (left), the distribution of the color-coded anisotropy vectors (0 to \( \pi/2 \)) indicating root orientation (middle), and the probability of the root orientation of individual roots (right). All individual roots of the respective control and stress treatment were grouped to calculate the average root orientation for two independent setups using circular statistics (Berens 2009; Uyttewaal et al. 2012) as shown in Figure 3A, B. In both cases the mean of the average root orientation in the stress group might suggest a transverse bias compared to the control group, which indicates a preferable alignment in the orientation of the stress axis. Although we should note that the bias was not significant with Student’s \( t \)-test, we recognized at least a few examples with strongly transverse bias, e.g., samples 2–4 in stress group in Figure 3C.

The contribution of root diameter allocation to the root system architecture

With no change in the shoot/root weight distribution, but a tendency for a root alignment near to the vibration axis, the question of an alteration in the root system architecture occurs. To analyze this, the relative contribution of roots grouped into different diameters was analyzed (Figure 4). While the relative contribution of lateral roots with a diameter of <0.03 cm showed a decrease in its contribution to the total root surface (Figure 4A) and root length (Figure 4B), their contribution to the total root volume decreased significantly (\( p=0.046 \)) (Figure 4C). Larger adventitious roots with a diameter >0.04 cm show the opposite effect with the tendency of an increased contribution to the total root length and the total root surface (Figure 4A, B), and a significant increase in their contribution to the total root volume with \( p=0.039 \) (Figure 4C). The root group consisting of lateral roots and small adventitious roots with >0.03 cm and <0.04 cm showed no obvious differences in all parameters.

Discussion

A tendency for a root orientation bias

The effects of mechanical stress on plants are various and are highly dependent on the plant species and the force and duration of the stress. Well analyzed examples are the effects of mechanical stress treatments on plant organ weight (Börnke and Rocksch 2018), and root system changes in response to mechanical stress from the soil composition (Potocka and Szymanowska-Pulka 2018). However, the effect of mechanical stress on the root system architecture (RSA) was mainly observed in older trees with an already established root system (Anderson et al. 1989; Coutts 1983; Ennos 2000).

A preferable strengthening of roots in a stress direction was predicted theoretically (Mattheck et al. 1997) and practically in windward and leeward stress directions in young trees in a wind tunnel (Stokes et al. 1995). In both experiments however, the stress is applied from one direction, e.g. the windward side, whereas the unidirectional vibrations in this article exerts the stress.
in one axis, e.g. the forward and backward movement of the shaker.

Here we used young cuttings of *P. nigra* with only a minimal root system at the beginning of the culture, which has a higher degree of plasticity compared to already established trees. Based on the more dispersed looks of the roots in the stress treatment (Figure 1C, D) and the tendency of the roots to have a transverse bias/align more frequently in the direction of the unidirectional vibrations (Figures 2, 3), it is proposed that the root orientation can be influenced by mechanical stress. However, it needs to be stated that both setups tested showed only a tendency, and no significant difference in a statistical sense. A reason might be too small sample numbers (n = 5 and n = 8 per group) and difficulties with the noise for the detection of thin roots.

**Thicker root diameter allocation**

A recent analysis of containerized *Quercus robur* and *Robina pseudoacacia* (Reubens et al. 2009) showed varying rooting responses to mechanical stress. While *R. pseudoacacia* increased the root biomass and an allocation to horizontal shallow roots, *Q. robur* allocated more to roots of the first order and deeper second order roots. The increased allocation to adventitious roots with a diameter larger than 0.04 cm in our analysis (Figure 4) and a respective tradeoff for the total contribution of smaller lateral roots with <0.03 cm suggests a comparable response of *P. nigra* to mechanical stress as shown for *Q. robur*.

**Mechanical reinforcement**

Upon mechanical stress, plants tend to mechanically reinforce the respective structures under stress to minimize the probability of damage. A reasonable hypothesis influenced by principles in mechanical engineering is the axiom of uniform stress (Mattheck 1998). This axiom states that the stress is averaged out, due to an increased growth in the stressed area, which reduces the stress in this region. We are aware that the axiom of uniform stress is controversial in the plant science area (Niklas and Spatz 2012; Slater 2016). However, taken the applicability to the whole tree level aside, the axiom is able to explain frequent observations in response to mechanical stress. An increased allocation to thicker roots, as observed here and elsewhere (Reubens et al. 2009) is in good agreement, since thicker materials are less prone to stress damage compared to thin roots.

The second factor that should be taken into account is the orientation of the roots. It is known that roots oriented in a stress direction are deformed to withstand mainly tensile and compressive force, since tree roots show respective deformations (Stubbs et al. 2019). A preferential distribution in the direction of wind stress is known for young trees (Stokes et al. 1995). However, since the trees analyzed in previous publications are fairly matured, it cannot be concluded if the adaption takes place after the roots were growing, or if the roots grow in this direction because of the mechanical stress. In this experiment we intended to analyze if young *P. nigra* are able to align their developing roots to the stress orientation as an adaptive response, though the quantitative analysis introduced here was not sufficient for this purpose.

**Conclusion**

Taken together, the roots of young *P. nigra* cuttings have an increased allocation to larger diameter roots. A statistically significant tendency for an increased root orientation nearer to the stress axis could not be observed. The observed modification can be seen as a plant strategy to mechanical reinforce their anchorage. Future research will focus on a more detailed analysis of the root distribution and confirm if mechanical stress can induce root growth in addition to altering the morphology of already existing roots.

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