Genetic diversity and performance of early life stages in natural and afforested populations of two important conifer trees in Syria

- Implication for conservation -

DISSERTATION

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Bäume sind Gedichte, die die Erde in den Himmel schreibt.

(Gibran Khalil Gibran, translated from Arabic)

Pine in the legends of Greeks

"*Pitys, a beautiful nymph, had two lovers, Pan and Boreas, she favored the former whereupon the latter. Boreas became jealous and threw Pitys against rocky ledge. She turned into a pine tree, and resin drops are her tears*".

(Mirov and Hasbrouck 1976)

Cypress in the poetry of Shakespeare

In ivory coffers I have stuffed my crowns;

In cypress chests my arras counterpoints;

Costly apparel, tents and canopies.

Shakespeare; In the Taming of the Shrew, II, i, 353

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SUMMARY

Per definition, afforestation is the process and outcome of planting trees to sites where forests do not naturally grow. Afforested tree species are often planted beyond their natural distribution range. Both from the view of conservation and forestry, afforested tree populations should have the genetic diversity and performance of their natural counterparts. In the course of the tree-breeding programs, trees for afforestation are raised following several consecutive steps and seedlings are produced under moderate conditions in nurseries. Selection process in nurseries differs from those in nature, which could depress species' ability to tolerate harsh conditions at planting sites. However, outplanted seedlings can experience selection that might select for higher stress tolerance in the individuals. Across the natural geographic range, natural tree populations are often large and continuous and the environmental conditions are optimal for the species. As a result, their genetic diversity is expected to be high and differentiation among populations is low. In the plantation area, populations are likely small and spatially isolated and the environmental conditions become unfavorable as the species distances from its natural zone. These circumstances are expected to lead to genetic drift, inbreeding and hampered gene flow. Consequently, afforested populations would be characterized by low genetic diversity and high among population genetic differentiation. In addition, as a consequence of afforestation practices, afforested populations would be either lost or acquired the ability to withstand stressful environmental conditions at planting sites.

This thesis is a compilation of three consecutive projects that evaluated the effects of afforestation practices on the genetic diversity and early-life-stage performance of *Pinus brutia* and *Cupressus sempervirens* in Syria. The comparative projects between natural and afforested populations in this thesis tested several main hypotheses.

(1) In the first project, I tested whether the afforested populations differ in their genetic diversity and differentiation from the natural populations. The results show that in case of *P. brutia*, the afforested populations maintained lower genetic diversity. This result can be interpreted by a decline in population size which might led to genetic drift and a potential inbreeding among closely related individuals. Contrary to our assumptions, afforested populations of *C. sempervirens* captured the same proportion of genetic diversity presented in the natural

populations. This finding suggests that the number of afforested population generations is still low to result in significantly lower genetic diversity in these afforestations. The more pronounced genetic differentiation being reported among the afforested populations in both species can be attributed to the spatial isolation and restricted gene flow.

(2) The second project tested whether natural and afforested populations show different germination performance in response to stress treatments under different temperature regimes. I carried out a germination experiment under controlled chamber conditions with fully crossed factorial design. Afforested *P. brutia* populations outperformed natural ones under drought stress levels at the intermediate and high temperatures. For *C. sempervirens*, natural populations showed better performance in response to higher salt stress at the intermediate temperatures. These findings suggest a high potential of afforested *P. brutia* populations to evolve and adjust their performance to changing climatic conditions. Such ability indicates that a strong selection pressure may have occurred after outplanting and the species might be locally adapted and developed into land races. In the case of *C. sempervirens*, the loss of salt-tolerance is likely due to the production of afforested seedlings under moderate climatic conditions in the nurseries.

(3) The third project investigated whether the seedling growth can be also impacted by the process of creating afforestations. I assessed performance in seedlings from *C. sempervirens* in a greenhouse experiment over four months under different temperature and moisture regimes. Seedlings from natural provenances outperformed afforested ones in all growth parameters under the conditions studied. This result can be explained by a potential inbreeding depression in small scattered afforested populations and indicates an enhanced growth in seedlings originated from favorable maternal environmental conditions.

In conclusion, the results of this thesis show that afforestation practices need to be altered in order to preserve genetic diversity in afforested populations. Practitioners should collect seeds from a wide variety of maternal phenotypes and mitigated the selection of mother trees on the bias of phenotypic superiority. In addition, further selection processes from seedling stock during nursery culture should be avoided. As the Randomly Amplified Polymorphic DNA are neutral selective markers, they are not appropriate to estimate population's adaptive potential or local adaptation. Therefore, it is recommended integrating studies of neutral DNA markers with those of *Quantitative Trait Loci* (QTLs). The results of this thesis strikingly underline that the differences between natural and afforested populations in genetic diversity and seed and seedling performance should be taken into account when selecting seed materials for future afforestation programs in Syria.

ZUSAMMENFASSUNG

Definitionsgemäß ist die Aufforstung der Prozess und das Ergebnis der Anpflanzung von Bäumen an Orten, an denen Wälder nicht natürlich wachsen. Die verwendeten Baumarten werden hierbeioft außerhalb ihres natürlichen Verbreitungsgebiets gepflanzt. Aus der Sicht des Naturschutzes und der Forstwirtschaft sollte in den aufgeforsteten Baumpopulationen die genetische Diversität und Leistungsfähigkeit der natürlichen Bestände erhalten bleiben. Im Zuge von Zuchtprogrammen werden die Bäume für die Aufforstung über mehrere Generationen hinweg g ezüchtet und die Keimlinge unter moderaten Bedingungen in Baumschulen produziert. Die Umweltbedingungen und damit Selektionsregimes in Baum-schulen unter-scheiden sich von den natürlichen Bedingungen. Dies kann die Fähigkeit der Keimlinge verringen, zu tolerieren. Jedoch können die g epflanzten Sämlinge Selektion in Richtung höherer Stresstoleranz erfahren. In den natürlichen g eografischen Verbreitungsgebieten sind die Populationen g roß und stetig und die Umweltbedingungen sind ein Optimum der Art. Infolgedessen ist die g enetische Vielfalt der natürlichen Baumpopulationen voraussichtlich hoch und die Differenzierung zwischen den Populationen gering. In aufgeforsteten Gebieten hingegen sind die Populationen wahrscheinlich klein und räumlich isoliert und die Umweltbedingungen sind ungünstig, wenn die Art weit entfernt von ihren natürlichen Vorkommen gepflanzt wurde. Diese Umstände führen voraussichtlich zu genetischer Drift, Inzucht und reduziertem Genfluss. Folglich sollten aufgeforstete Populationen durch niedrige genetische Diversität und eine hohe g enetis-che Differenzierung zwischen Population charakterisiert sein. Zusätzlich, als Folge des Aufforstungsprozesses, können aufgeforstete Populationen entweder verloren gehen oder sie erwerben ihre Fähigkeit, die stressigen Umweltbedingungen am Ort der Pflanzung zu tolerieren.

Diese Dissertation besteht aus drei Projekten, die die Auswirkungen des Aufforstungsprozesses auf die genetische Diversität und die Wuchsleistung der frühen Lebensphasen von *Pinus brutia* und *Cupressus sempervirens* in Syrien bewerten. In vergleichenden Projekten zwischen natürlichen und bewaldeten Populationen wurden in dieser Arbeit mehrere Hypothesen getestet.

(1) Im ersten Projekt wurdeuntersucht, ob die aufgeforsteten Population sich in ihrer genetis-chen Diversität und Differenzierung von den natürlichen Populationen unterscheiden.

Die Ergebnisse zeigen, dass die aufgeforsteten Populationen im Falle von *P. brutia* eine niedrigere genetische Diversität aufweisen. Dieses Ergebnis kann durch einen Rückgang der Populationsgröße erklärt werden, derzu genetischer Drift und die Möglicherweise zu Inzuchtdepression nach Kreuzung nahe verwandter Individuen führen kann. Im Gegensatz zu den Annahmen wiesen die aufgeforsteten Populationen von *C. sempervirens* den gleichen Anteil genetischer Vielfalt wie die untersuchten natürlichen Populationen auf. Dieser Befund legt nahe, dass trotz der geringen Populationsgröße, die aufgeforsteten Population noch zu wenige Generationen durchlaufen haben, um eine bedeutende Reduktion der genetischen Diversität zu erfahren. Die deutlichere genetische Differenzierung zwischen den aufgeforsteten Populationen kann bei beiden Arten räumlicher Isolation und eingeschränktem Genfluss zugeschrieben werden.

(2) Das zweite Projekt untersucht, ob die natürlichen und aufgeforsteten Populationen unterschiedlichen Keimungserfolg in Reaktion auf Stressbehandlungen zeigen. Es wurde ein Keimexperiment unter kontrollierten Bedingungen und mittels eines vollfaktoriellen Designsdurchgeführt. Aufgeforstete *P. brutia* Populationen übertrafen die natürlichen Populationen unter den Trockenstress an den hohen und mittleren Temperaturen. Für *C. sempervirens* zeigten die natürlichen Populationen bessere Leistung als Reaktion unter starken Salzstress an den mittleren Temperaturen. Diese Befunde deuten auf ein hohes Potenzial aufgeforsteter Populationen von *P. brutia* ihre Leistung veränderten klimatischen Bedingungen anpassen zu können. Diese Fähigkeit zeigt, dass ein starker Selektionsdruck nach der Pflanzung auftreten kann und dass die Art lokal angepasst zu sein scheint und im Begriff ist neue Rassen zu entwickeln. Im Fall von *C. sempervirens* ist der Verlust der Salztoleranz wahrscheinlich auf die Züchtung der Jungpflanzen unter moderaten klimatischen Bedingungen in den Baumschulen zurück zu führen.

(3) Das dritte Projekt untersuchte, ob das Wachstum der Keimlinge auch durch Prozesse im Verlauf von Aufforstungen beeinflusst werden kann. Es wurde die Leistung der Sämlinge von *C. sempervirens* in einem Gewächshausversuch über vier Monate unter verschiedenen Temperaturen und Bewässerungsregimesermittelt. Die Sämlinge aus natürlichen Provenienzen übertrafen die aus aufgeforsteten Populationenin allen Wachstumsparametern unter den untersuchten Bedingungen. Dieses Ergebnis kann durch Inzuchtdepression in kleinen

zerstreuten aufgeforsteten Populationen erklärt werden und es deutet auf ein erhöhtes Wachstum von Keimlingen, die aus günstigen mütterlichen Umwelt bedingungen stammten. Abschließend zeigten die Ergebnisse dieser Arbeit, dass die Maßnahmen zur Aufforstung verändert werden müssen, um die genetische Diversität in aufgeforsteten Populationen zu bewahren.

In der Praxis sollten die Samen von einer Vielzahl mütterlicher Phänotypen gesammelt werden. Darüber hinaus sollten starke Selektionsprozesse während der Zuchtin Baumschulen vermieden werden. Der in dieser Arbeit verwendete Marker (RAPD) ist als neutral selektiver Markers nicht geeignet, um Informationen über das adaptive Potenzial einer Population oder zum Schätzen der lokalen Anpassung zu erhalten. Daher wäre es besser, Studien mitneutralen DNA-Mark-ern und der Analyse von sogenannten *Quantitative Trait Loci* (QTL) zu kombinieren. Die Ergebnisse dieser Arbeit machen deutlich, dass die Unterschiede zwischen natürlichen und aufgeforsteten Populationen bezüglich genetischer Diversität und Leistung der Samen und Keimlinge bei der Selektion des Samenmaterials für spätere Aufforstungsprogramme in Syrien in Betracht gezogen werden sollten.

الملخَّص

التشجير الحراجي: هو عملية و نتيجة زراعة الأشجار في مواقع لا تنمو فيها الغابات بشكل طبيعي. في أعمال التشجير الحراجي، غالبا" ماتزرع الأنواع الشجرية خارج نطاق توزعها الطبيعي. من وجهة نظر بيئية و حراجية، يجب ان تحتوي المجموعات الشجرية المزروعة على التنوع الوراثي و الإنبات و نمو الغراس بشكل مماثل لنظيرتها الطبيعية. في برامج التربية، يتم تربية الاشجار المعدَّة للتشجير من خلال خطوات متعددة و متتابعة ويتم إنتاج الغراس في المشاتل الحراجية تحت ظروف بيئية معتدلة نسبياً. عملية الاصطفاء في المشاتل الحراجية تختلف مما هو عليه الحال تحت ظروف النمو الطبيعية، مما يؤثر سلباً على قدرة النوع على تحمل الظروف البيئية القاسية في مواقع التشجير. من ناحية أخرى، فإنَّ زراعة الغراس تحت تلك الظُّروف القاسية قد يعرّضها لعملية اصطفاء طبيعي مما قد ينتج عنه أفراد قادرة على تحمل الإجهاد. ضمن المجال الطبيعي لتوزع النوع، غالباً ما تكون المجموعات الشجرية الطبيعية واسعة و مستمرة الانتشار، باللإضافة لذلك فإن الظروف البيئية تكون في حدِّها الأمثل بالنسبة للنوع. كنتيجة لما ذكر آنفاً, فإنَّه من المتوقع ان يكون التنوع الوراثي لتلك المجموعات عالياً بينما تكون الفروقات الوراثية بينها ضئيلة. في مناطق التشجير، غالباً ماتكون المجوعات المشجرة صغيرة و متباعدة مكانياً و تصبح الظروف البيئية غير ملائمة كلما ابتعد النوع عن منطقة انتشاره الطبيعية. هذه الظروف قد تؤدي بدورها إلى انحراف وراثي و توالد داخلي بالإضافة إلى إعاقة أو حصر للتدفق االجيني بين المجموعات. بالتالي فإنَّ المجموعات المشجرة ستتصف بقلة التنوع الوراثي وبأنَّ الفروقات الوراثية بين المجموعات تكون كبيرة. بالإضافة إلى ذلك، و كنتيجة لعمليات التشجير فإنَّ المجموعات المشجرة ستكون إما اكتسبت أو فقدت القدرة على تحمل الظروف البيئية القاسية في مواقع التشجير.

هذه الأطروحة مؤلفة من ثلاثة مشاريع أو مواضيع متتابعة لتقييم تأثير إنشاء المشجرات على التنوع الوراثي و الأداء في مراحل النمو المبكرة في الصنوبر البروتي و السرو دائم الخضرة في سوريا. المواضيع المقارانائية بين المجموعات الطبيعية و المشجر اختبرت عدة فرضيات.

أولاً(١): في المشروع الأول، قمت باختبار فيما إذا كانت المجموعات المشجرة تختلف في تنوعها الوراثي و تبدي اختلافات وراثية أكبر فيما بينها مقارنةً مع نظيراتها الطبيعية. أظهرت النتائج أنَّه بالنسبة للصنوبر البروتي، المجموعات المشجرة احتوت على تنوع وراثي أقل مقارنة مع المجموعات الطبيعية. هذه النتيجة يمكن أن تفسر بنقص في حجم المجموعات المشجرة، الأمر الذي يؤدي بدوره إلى انحراف وراثي واحتمال حدوث تزاوج بين أفراد مرتبطين بصلة القرابة. بشكل مخالف لتوقعاتنا، احتوت المجموعات المشجرة من السرو دائم الخضرة على نفس التنوع الوراثي الموجود في المجموعات الطبيعية. تشير هذه النتيجة إلى أنَّ عدد أجيال المجوعات المشجرة قد لايزال منخفظاً جداً لكي يُحدث نقصاً في التنوع الوراثي لتلك المجموعات. التمايز الوراثي بين المجموعات المشجرة من كلا النوعين كان أكثر وضوحاً مما هو عليه الحال بين المجموعات الطبيعية. يمكن أن يعزى ذلك إلى التباعد المكاني بين المجموعات المشجرة وتقييد تدفق الجينات فيما بينها.

ثانياً (٢): اختبرت في المشروع الثاني فيما إذا كان المجموعات الطبيعية و المشجرة ستظهر اختلافات في الإنبات بالاستجابة إلى معاملات إجهادية مختلفة. نفذت تجربة الإنبات تحت ظروف محكمة في حجرات الإنبات وتصميم عاملي كامل التداخل. تفوقت المجموعات المشجرة من الصنوبر البروتي في إنباتها تحت ظروف الإجهاد الجفافي ودرجات الحرارة المعتدلة و المرتفعة على المجموعات الطبيعية. تشير هذه النتيجة إلى قدرة عالية للمجموعات المشجرة من الصنوبر البروتي على التطور وتعديل أدائها مع تغير الظروف البيئية. تدل هذه القدرة العالية على أن المجوعات المشجرة من الصنوبر البروتي ربما تكون قد تعرضت لاصطفاء طبيعي شديد بعد الزراعة في الظروف الطبيعية لمواقع التشجير وأن النوع ربما يكون قد تكيف محلياً و تطور إلى عروق أرضية. بالنسبة للمجموعات المشجرة من السرو دائم الخضرة، فإنَّ نقص القدرة على تحمل الملوحة مقارنة مع المجموعات الطبيعية هو على الأرجح بسبب إنتاج الغراس تحت ظروف بيئية معتدلة في المشاتل الحراجية.

ثالثاً (٣): استقصيت في المشروع الثالث فيما إذا كان نمو الغراس أيضاً قد تأثر بعملية إنشاء المشجرات. قمت بتقييم أداء غراس السرو دائم الخضرة بزرعها في بيت زجاجي تحت ظروف درجات حرارة و سقاية مختلفة. تظهر نتائج هذا البحث ن الغراس الطبيعية تفوقت في نموهاعلى الغراس المشجرة تحت ظروف درجات الحرارة والرطوبة المختلفة. يمكن أن تفسر هذه النتيجة بتزاوج محتمل بين الأقارب و الذي تزداد إمكانية حدوثه كلما كانت المجموعات المشجرة صغيرة و متناثرة، كما تدل على زيادة في نمو الغراس المنحدرة من أشجار أمهات نمت في ظروف بيئية مثلى.

أخيراً، تظهر نتائج هذه الأطروحة أنَّ عملية التشجير تحتاج أن تعدل من أجل حفظ التنوع الوراثي في المجموعات المشجرة. ينبغي على الحراجيين أن يقوموا بجمع البذور من قاعدة واسعة من أمهات البذور و تخفيف الاختيار على أساس التفوق المظهري. بالإضافة إلى ذلك، ينبغي خلال عملية التربية في المشاتل الحراجية تجنب اختيار الغراس الأكبر حجماً فقط للتشجير. بما أنَّ تقنية التضخيم العشوائي للحمض النووي متعدد الأشكال هي انتقائية محايدة، فإنها غير مناسبة لتقدير إمكانية التكيف عند النوع أو تحديد التكيف مع الظروف المحلية.ولذلك، فمن المستحسن دمج الدراسات المتعلقة بواسمات الحمض النووي المحايدة مع تقنية *Quantitative Trait Loci* (QTLs). نتائج هذا البحث تؤكد بشكل لافت للنظر أنَّ الاختلافات بين المجموعات الطبيعية و المشجرة من الصنوبر البروتي و السرو دائم الخضرة في التنوع الوراثي و في إنبات البذور و كذلك نمو الغراس ينبغي أن تؤخذ بعين الاعتبار عند اختيار مواد البذور من أجل برامج التشجير المستقبلية في سوريا.

Chapter 1 GENERAL INTRODUCTION

The role of conifer trees in cultivation

Since pre-historic eras, conifers have played a key role in human civilization. The East Mediterranean and Mesopotamian populations imported large quantities of wood from Syria (Meiggs 1982). Pine and cypress wood was used by Assyrian, Phoenician and Minoans for construction purposes (Holmes 1900; Thirgood 1981; Hepper 1990; Le Naitre 1998). There were also many famous parks and arboreta in Babylon and Persia (Meiggs 1982). Pines may have been grown in these parks and gardens; however, cedar and cypress were of the most popular trees as ornamentals (Le Maitre 1998). Beside their importance as natural forests in the East Mediterranean, *Pinus brutia* and *Cupressus sempervirens* are widely used in cultivation programs across and outside their natural range (Borelli 2002; Giovanelli and De Carlo 2007; Baldi et al. 2011). Due to the long history of exploitation that goes back to the ancient times, the natural distribution range of these species has largely declined (Farjon 2008).

In the 14th century, growing concern about the magnificence and state of forest resources led to large scale of afforestation mostly with conifer species (Le Maitre 1998). Afforestation is defined as the act of establishing forests through planting seedlings on non-forested land (Reyer et al. 2009). Conifers are among the most genetically diverse organisms (Hamrick et al. 1992). Genetic diversity, for instance, permitted pines to evolve in response to environmental change over the last 200 million years (Miller 1993). The combination of these features makes conifers an ideal species group for plantation programs (Ledig 1998). In Syria, the northern part of the country was still well forested at the end of 19th century (Meiggs 1982). However, the natural forest area has drastically declined from 30% to only about 2% of the country area over the last century (FAO 2003). In the face of such depletion, Syria started plantation efforts in the 1950s (Ghazal 2008). Nevertheless, the high commission for afforestation was established only in 1977, with an annual work plan of 20,000 ha for afforestation development (Nahal and Zahoue 2005). *Pinus brutia* and *C. sempervirens* are the main species for the national afforestation programs in Syria (IPGRI 2001; Nahal and Zahoue 2005), where they are afforested throughout different bioclimatic regions across and beyond their natural range.

Genetic diversity and differentiation in natural *vs*. afforested populations

The genetic structure of wild growing populations has evolved over many generations via the processes of selection, migration, mutation, and random genetic drift (El-Kassaby and Ritland 1996). Across the natural range of a forest species, populations are often large and continuous and gene flow is extensive which enhance genetic diversity and prevent genetic differentiation (White et al. 2007). However, the domestication of wild species bears the danger that rates of genetic change are more affected than could be expected under natural evolutionary processes (El-Kassaby 1995).

In the process of breeding tree species, each of the consecutive breeding stages can contribute to erosion in the genetic diversity. These stages are (i) phenotypic selection; (ii) breeding programs with its associated activities; (iii) seed production; (iv) seedling production; (v) the establishment of plantations (El-Kassaby 1995; El-Klassaby and Namkoong 1995, Fig. 1). The process of selection based on desired phenotypic traits inevitably reduces the effective population size. In turn, a small number of inbreeding individuals can decrease genetic diversity through self-pollination and inbreeding (Wang et al. 1999). Furthermore, the selection process can alter the allele frequencies of few traits that are considered important and consequently, cause bottleneck effects (El-Kassaby and Thomson 1996; Bagnoli et al. 2009). In addition, the multiple sowing followed by thinning in nurseries favors certain parents (Edwards and El-Kassaby 1996) and can introduce directional selection, thus reducing the expected genetic variation among seedlings (El-Kassaby 1995; El-Kassaby and Thomson 1996). Several studies have shown that the amount of genetic variation present in natural populations was retained or even increased during the phenotypic selection stage in some conifer species (Knowles 1985; Chaisurisri and El-Kassaby 1994; El-Kassaby et al. 1994). In contradiction to these results, reduction in genetic diversity in the phenotypic tree-improvement selections reported in other researches (Cheliak et al. 1988; Stoehr and El-Kassaby 1997; Rajora 1999).

In order to assess the levels of genetic diversity within planted forests after generations of the establishment, Medri et al. (2003) and Stefenon et al. (2008) found no differences in genetic diversity between natural and planted populations of *Pinus contorta* and *Araucaria angustifolia* respectively. However, significant reduction in genetic diversity was reported in planted populations of *Picea abies* (Gömöry 1992).

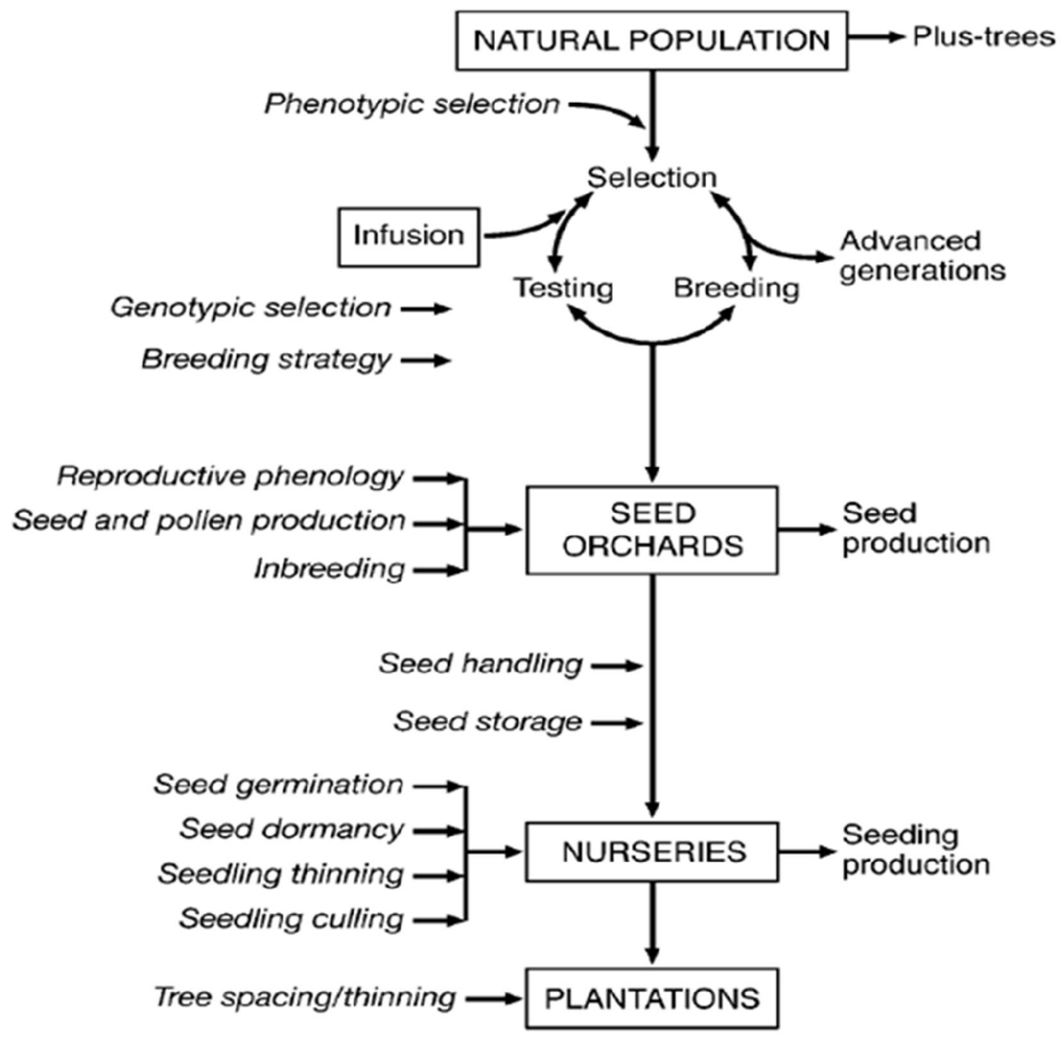

Fig. 1 A diagram describing the stages of the tree improvement delivery system and the associated activities where genetic diversity may be lost (El-Kassaby 2000; see, White et al. 2007, p 282 for copyrights).

In summary, the occurrence of population bottleneck, inbreeding and the prevention of gene flow among small and spatially insolated populations can cause a successive loss of heterozygosity and increase genetic differentiation among populations (Ellstrand and Elam

1993; Young et al. 1996). Thus, monitoring genetic diversity in planted forests can provide information on whether man-made plantations may contribute to the conservation of the species' gene pool (Bergman and Ruetz 1991).

Performance of early life stages in natural and afforested populations

The fitness and adaptation in a target species can be affected in several ways. First, in small and spatially isolated tree populations, inbreeding depression can occur and affect performance of an offspring (Sorensen 1969; Sorensen and Miles 1982). For instance, in Pinaceae, inbreeding depression generally results in higher embryo abortion, lower germination success and reduced growth (see, Salzer and Gugerli 2012 and references herein).

Second, raising seedlings under moderate environmental conditions in the nurseries can also pose another constraint on tree fitness. The biotic and abiotic properties are more homogeneous than the conditions in natural forests (Rajora 1999). In this context, in the juvenile stage, planting gives less opportunity for natural selection because initial density is lower and juvenile mortality is reduced (Lefévre 2004). However, as a species is established in a stressful environment, strong natural selection will operate and some seedlings will survive and grow remarkably well (Ledig and Kitzmiller 1992). As a sequence, local land races may begin to evolve in the new edaphic and climatic conditions (White et al. 2007). The developed land races may show superiority in their growth over the natural provenances (Finkeldey and Hattermer 2007).

Third, in conifer species in particular, the maternal environment maternal effects defined as the direct contribution of the maternal environment to the offspring phenotype (Roach and Wulff 1987) can strongly influence fitness parameters (Yakovlev et al. 2012). In this regard, for instance, the species acquires cold resistance only during embryo development under lower temperature in the field and lose its adaptedness owing to raised temperatures during seed maturation (Finkeldey and Hattermer 2007). However, it was shown that the favorable environmental conditions can permit the species to perform better than stressful maternal environment (Kärkkäinen et al.1999; Cendán et al. 2013; Zas et al. 2013).

Last but not least, the performance of an offspring could also be affected by the geographic origin of seed material (White et al. 2007; Donohue et al. 2010). Bekessy et al. (2002) tested provenances of *Araucaria araucana* in the greenhouse experiment and found much higher drought resistance in provenances from the drier regions.

Seed weight is strongly influenced by environmental maternal conditions (Clair and Adams 1991; Linkies et al. 2010), such water stress (Castro 1999). This in turn may influence germination success and seedling growth (Castro et al. 2006; Tiscar and Lucas 2010; Cendán et al. 2013). In areas with extreme environmental conditions, like the Mediterranean region, the risks that afforestation practice bears are particularly pronounced. By raising and selecting seedlings in nurseries, tree species might lose their ability to successfully germinate and establish in stressful conditions, such as under drought and salt stress (Navarro et al. 2006; Plourde et al. 2009). So far, scarce reports are available on a comparison between seed germination and seedling growth between natural and afforested populations in conifer species. Zhu et al. (2006) pointed out that in *Pinus sylvestris* var. *mongolica*, seeds from natural populations outperformed plantation seeds in their germination under reduced water potentials suggesting that natural seeds are more resistant to drought stress. This finding was interpreted by adaptation of the natural forests to their harsher environmental conditions. Likewise, compared to naturally regenerated seedlings of *Pinus halepensis*, nursery grown stock had much more biomass (Oliet et al. 2002).

Study species

Pinus brutia Ten. subsp. *brutia*

The genus *Pinus* comprises about 100 species, varieties and hybrids of evergreen trees and rarely shrubs (USDA 1948; Krugman and Jenkinson 2008). *Pinus brutia* has a large ecological adaptability, its tolerance to sub-humid environments, high temperatures and drought make it suitable for cultivation even out its natural range (Borelli 2002). Furthermore, these characteristics make it an irreplaceable forest species within the fragile Mediterranean ecosystems (Fischer et al. 2008). In Syria, the natural forests of this species distribute from the Thermo-Mediterranean to Supra-Mediterranean but mostly occur in the Meso or Eu-Mediterranean; in addition, the species forms natural stands in the semi-arid region (Nahal and Zahoue 2005).

Pines are monoecious diploid organisms (2n = 24) (Ledig 1998). Depending on elevation and temperature, flowering in *P. brutia* occurs between March and May (see, Boydak 2004). Seeds are arranged in cones and ripe from January to March of the next year (Krugman and Jenkinson 2008). Reproductive structures in *P. brutia* first form when the trees are 7 to 10 years old with one year between cone crops (see, Boydak 2004). In this species, some or all of the mature cones remain closed for several to many years, or they open on the tree at irregular intervals (Krugman and Jenkinson 2008).

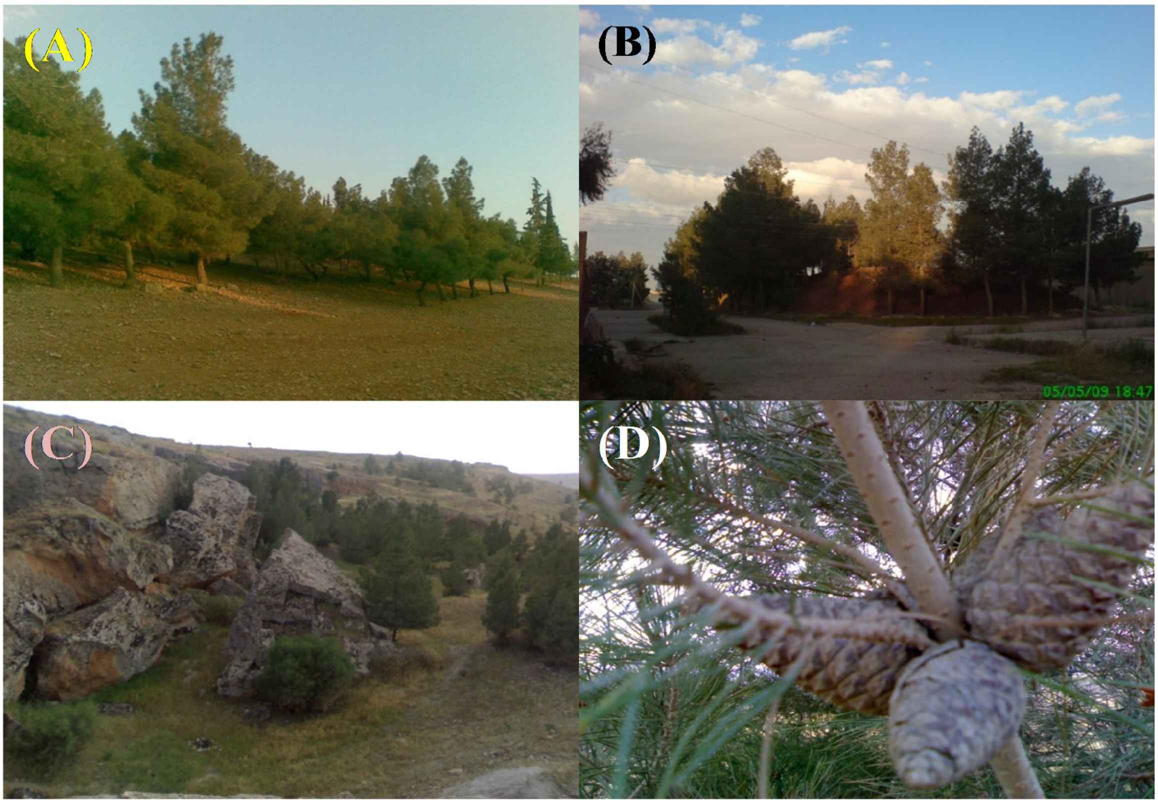

Fig. 2 Afforestation of *Pinus brutia* and *Cupressus sempervirens*. A: Mixed afforested stand of the two species; B: Afforested *Pinus brutia* population; C: Afforested *Pinus brutia* population on Um Assarj mountain (Manbij); D: Characteristic cone positioning in *Pinus brutia* (a key trait in distinguishing *Pinus brutia* from *Pinus halepensis*). Photos by Batoul N. Al-Hawija (2009).

Seed dispersal occurs throughout the year, with most seeds dispersed between July and December (Boydak 2004). Seeds germinate in nature throughout the rainy season if temperatures are suitable, often in massive numbers in spring and, more rarely, in the autumn (see, Boydak 2004; Thanos et al. 1989; Thanos and Doussi 2000).

Germination percentage is relatively high in *P. brutia*, where average germination is 80% (Piotto et al. 2003).

Cupressus sempervirens L. var. *horizontalis* (Mill.)

The genus *Cupressus* comprises about 15 species of evergreen trees or shrubs (Krugman and Jenkinson 2008). In Syria, wild growing *C. sempervirens* occurs as discrete populations along the coastal mountains and afforested across wide range of bioclimatic gradients (Ghazal 2008). *Cupressus sempervirens* can withstand long, warm, dry summers, and cold, fairly harsh winters (Brofas et al. 2006), and his resilient nature makes it irreplaceable in arid and poor soils (Teissier du Cros 1999; Manescu et al. 2011).

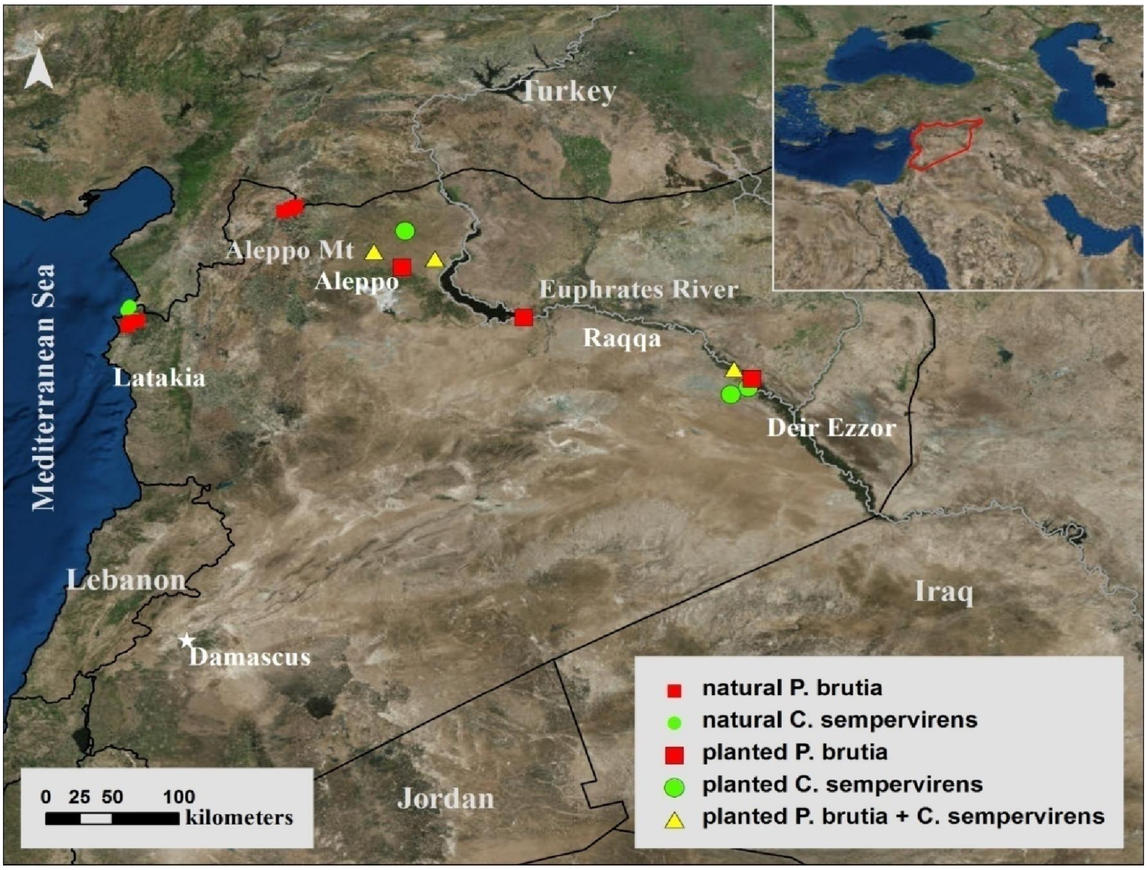

Fig. 3 Sample locations of the natural and afforested populations of *Pinus brutia* and *Cupressus sempervirens* in Syria.

It is monoecious diploid (2n = 22) species (Mehra and Hunziker 1956). *Cupressus sempervirens* L. first flowering occurs at the age of 3-4 years and the reproductive cycle extends over 3 years, with initiation and differentiation of flowers in the first, pollination and cone growth in the second, seed maturation and dispersal in the third year or in the first months of the fourth year (Giannini et al. 1999).

Cones can remain for as long as 20 years on the tree (Praciak et al. 2013). If the seeds are kept in airtight containers at 3 °C and 5-6% moisture content, they will retain viability for 7-20 years (Piotto et al. 2003). Seeds are often heavily contaminated with mold and bacteria during stratification and germination (Krugman and Jenkinson 2008). Germination percentage of *C. sempervirens* averaged only 20-40% (Piotto et al. 2003). One of the major problems in the production of cypress seedlings is the poor quality of the seed material, which has been attributed to the high percentage of empty seeds normally present in seed lots (Spyroglou et al. 2009).

Study objectives

The present thesis analyzes the effects of afforestation process on the genetic diversity and performance of *Pinus brutia* and *Cupressus sempervirens*. I contrasted wild growing populations in the natural range of the target species with afforested populations planted outside the natural distribution range of the species in Syria (Fig. 3). In particular, the study asks two questions: 1) Do afforested populations represent the full genetic diversity found in natural stands? The answer to this question may help foresters to evaluate to what extent their breeding practice erodes genetic diversity and to what extent their afforestation can conserve the species' gene pool. 2) Does afforestation lead to lower seed and seedling performance under environmental stress? The findings can predict whether afforested tree individuals can sustain viable populations under the Mediterranean climate. I addressed these questions in three studies with several hypotheses (see, Fig. 4 for the main hypotheses of the thesis).

The first study **(Chapter 2)** compared the genetic diversity between natural and afforested populations of the study species. Here, I investigated the hypotheses: (1) That afforested populations are genetically less diverse in terms of expected heterozygosity (H_e) and percentage of polymorphic loci (*PPL*) than natural populations; (2) That afforested populations exhibit more genetic differentiation among them than natural populations.

The second study **(Chapter 3)** investigated the differences in seed germination performance between natural and afforested population types of both species in response to simulated drought and salt stress. The study tested the following hypotheses: (1) Compared to natural populations, afforested populations have either lost or acquired their tolerance for cold and high temperatures as well as for drought and salt stress; (2) Germination response may be influenced by seed weight, i.e. heavier seeds will exhibit higher germination; and (3) Germination responses in natural and afforested populations might be influenced by their climatic provenance, i.e. mean annual temperature and annual precipitation sum at the seed collection sites. Specifically, I expected that populations located in more arid regions show enhanced germination.

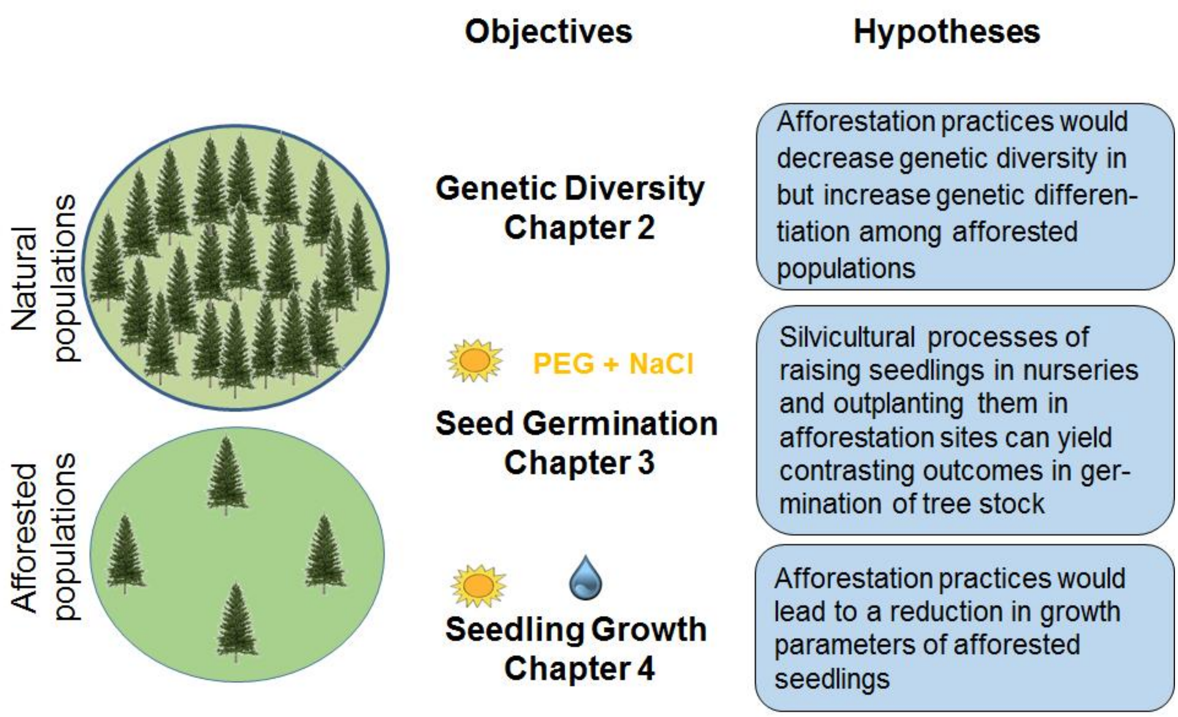

Fig. 4 Summary of the objectives and the main hypotheses investigated in this thesis. Trees here symbolize the population types. Natural populations are large and continuous; afforested populations are small and spatially isolated. PEG: Polyethylene Glycol; NaCl: Sodium chloride.

In the third study **(Chapter 4)** I examined seedling performance in natural and afforested population types of *C. sempervirens* under different temperature and moisture regimes.

Unfortunately, we lacked seed material from *P. brutia* in this study. I hypothesized that seedling growth would be reduced: a) For afforested compared to natural populations; b) Under higher compared to lower temperatures; c) Under drier compared to moist conditions. In addition, I expected that d) Natural and afforested populations would react differently to the different climatic conditions.

Chapter 2 GENETIC DIVERSITY

Genetic comparison between natural and planted populations of *Pinus brutia* and *Cupressus sempervirens* in Syria

Batoul N. Al-Hawija, Viktoria Wagner and Isabell Hensen

TURKISH JOURNAL OF AGRICULTURE AND FORESTRY (2014) 38: 267-280

ABSTRACT

There is wide consensus that ongoing deforestation contributes to global warming and poses a threat to species diversity. Less understood is whether the practice of creating plantations might also erode genetic diversity and undermine the genetic structure of tree populations. We tested these hypotheses in natural and planted populations of *Pinus brutia* Ten. subsp. *brutia* and *Cupressus sempervirens* L. var. *horizontalis* (Mill.), two important forestry species in the Mediterranean region. We used plant material from three different bioclimatic regions in Syria. Using RAPD markers, we evaluated the genetic diversity and structure of 12 populations of *P. brutia* (six natural, six planted) and nine populations of *C. sempervirens* (three natural, six planted).

Expected heterozygosity (H_e) and percentage of polymorphic loci (*PPL*) were high in both species (*P. brutia*: $H_e = 0.241$, *PPL* = 81.2%; *C. sempervirens*: $H_e = 0.241$, *PPL* = 78.8%). In accordance with our assumptions, plantations of *P. brutia* manifested significant reduction in mean genetic diversity; this result, however, was not revealed in *C. sempervirens*. Analysis of molecular variance (AMOVA) demonstrated that the genetic structure of plantations differed from that of natural populations. Interestingly, plantations of both species harbored more genetic differentiation among them than natural populations. The partitions created by AMOVA also showed a significant differentiation between two groups, natural populations versus plantations in the two species, and among bioclimatic regions only in *C. sempervirens*. This result was corroborated by cluster analyses, which indicated a closer relationship among populations from the same geographic region. Genetic distance was positively related to geographic distance only in natural populations of *P. brutia*.

Plantations in our research showed a significant reduction in genetic diversity, particularly in *P. brutia*, and stronger among-population genetic differentiation compared to natural populations. We recommend that forest management incorporates genetic diversity and differentiation as an important criterion for selecting appropriate tree stock material.

KEYWORDS: Deforestation, Genetic differentiation, Genetic diversity, Genetic structure, Plantations, Syria.

Chapter 3 SEED GERMINATION

Germination differences between natural and afforested populations of *Pinus brutia* and *Cupressus sempervirens*

Batoul N. Al-Hawija, Viktoria Wagner, Monika Partzsch and Isabell Hensen

SILVA FENNICA (2014) 48(4): 1-18

ABSTRACT

In afforestation, the silvicultural processes of raising and outplanting seedlings might yield contrasting outcomes in the performance of tree stock. Moderate nursery conditions may select against stress tolerance whereas planting seedlings in stressful environments at afforestation sites might select for higher stress tolerance compared to natural populations.

We contrasted germination performance between natural and afforested populations of *Pinus brutia* Ten. subsp. *brutia* and *Cupressus sempervirens* L. var. *horizontalis* (Mill.), under different stress treatments. We collected seeds from natural populations and from afforested populations outside the natural distribution range, in Syria. We implemented cold, intermediate and hot temperature regimes (8/4 °C, 20/10 °C and 32/20 °C), cold stratification, drought stress (-0.2 and -0.4 MPa), salt stress (50 and 100 mMol/l) and de-ionized water as a control. Additionally, we tested the effects of seed weight and climatic conditions on seed germination.

In general, the intermediate temperatures were optimal for both species and population types. In *P. brutia*, afforested populations outperformed natural ones under both drought stress (-0.2 and -0.4 MPa) at intermediate and hot temperatures. By contrast, in *C. sempervirens*, cold stratification at all temperatures as well as the higher salt stress at the intermediate temperatures significantly decreased seed germination of afforested populations compared to the natural ones. Seed weight did not significantly affect germination percentage in both species. Germination percentage significantly negatively related to annual precipitation at seed collection sites in *P. brutia,* and to annual temperature in *C. sempervirens*.

We infer that silvicultural process led to divergent outcomes in our species: local adaptation to drought stress and hot temperatures in afforested *P. brutia* populations and a loss in salt-stress tolerance in *C. sempervirens*.

KEYWORDS: Cold stratification, Drought stress, Nursery, Local adaptation, Salt stress, Silviculture, Syria.

Chapter 4 SEEDLING GROWTH

Performance of seedlings from natural and afforested populations of *Cupressus sempervirens* under different temperature and moisture regimes

Batoul N. Al-Hawija, Susanne Lachmuth, Erik Welk and Isabell Hensen

PLANT SPECIES BIOLOGY (2015) 30(4): 257-271

ABSTRACT

For successful afforestation programs seed quality is crucial, but seedlings are susceptible to climatic stress. Therefore, to improve afforestation success it is necessary to compare performance of seedlings from natural and cultivated populations under different climatic conditions. We investigated growth performance in seedlings of three natural and four afforested Syrian *Cupressus sempervirens* L. populations under different temperature and moisture regimes. A "warm" climate chamber approximately simulated current mean annual temperatures (day/night: 20/10 °C) while a "hot" chamber simulated an average increase of 5°C (day/night: 25/15 °C). Seedlings were irrigated twice (drier) or thrice (moist) weekly. Seedlings from natural provenances outperformed those from afforested stands in all growth variables in both chambers. In the warm chamber, root length and biomass were not affected by irrigation for both population types, but shoot height decreased for afforested seedlings under drier treatment while it slightly increased in natural seedlings. In the hot chamber, shoot height decreased but root length and biomass increased for population types under the drier treatment. Comparison between the two chambers showed that under the drier treatment shoot height and biomass decreased at higher temperatures, but root length and biomass were not significantly different. The same response to higher temperatures was observed under the moist treatment, but root biomass decreased too. Our results emphasize the necessity to protect the remaining natural forest of *C. sempervirens* in Syria and recommend systematic collection of seed material from natural stands for afforestation programs. This might also hold for *ex situ* cultivation of retrieving rare and endangered plant species.

KEYWORD: Afforestation, Climate, *Cupressus sempervirens*, Irrigation, Seedling, Syria.

Chapter 5 SYNTHESIS

GENERAL DISCUSSION

The outcomes of comparing the genetic diversity between natural and afforested populations in this thesis (Fig. 5) suggests that in case of *P. brutia*, only few mother trees might have contributed to the afforestation stocks (Gauli et al. 2009). In addition, the afforested populations might have undergone further selection throughout the domestication stages by keeping only the largest seedlings for outplanting that may have led to a bottleneck effect (El-Kassaby 1995). The finding in *C. sempervirens* might be attributed to the low number of generations in the afforested populations (El-Kassaby and Ritland 1996; Stefenon et al. 2008); so it might be assumed that a significant depletion may still occur in the following generations (Lowe et al. 2005). However, afforested populations of the two species exhibited stronger genetic differentiation among them compared to the natural populations, the finding that likely resulted from small population sizes and hampered gene flow among spatially isolated populations (Bucci et al. 1997; Wu et al. 1999).

The comparison of germination performance revealed that in at least two generations after the establishment and the reproduction in the stressful conditions in afforestation sites, a local adaptation in the afforested populations of *P. brutia* to more hot and dry conditions and the development of land races may have occurred. This result is in line with our other finding that populations originated from areas with less precipitation exhibited enhanced germination. Therefore, we can assume that the strong selection acting at the juvenile stage experienced by trees with low survivorship may allow rapid adaptation to changing climates (Kremer et al. 2012). The fast formation of land races indicates a high potential of these populations to evolve and adjust their performance to changing climatic conditions (Bigras and Colombo 2000). In this context, species can tolerate a specific condition only when this condition is prevailed during embryo development. This means that a species might lose its adaptation to hot conditions in the field if temperatures were moderate during seed maturations (Finkeldey and Hattermer 2007). Skrøppa and Johnsen (2000) found that offspring of *Picea abies* possessed considerably less tolerance to frost if the seeds were produced in a heated greenhouse. Moreover, in *Picea glauca*, seedlings from mother trees grown at colder conditions have been shown to be more frost hardy (Stoehr et al. 1998). This thesis used neutral markers to assess genetic diversity. Neutral marker studies may have useful application in conservation of a species (Ennos 1996).

However, they are not appropriate as a tool for evaluating population's adaptive potential or to estimate local adaptation (Bekessy et al. 2002). Hence, integration of neutral markers and quantitative trait loci (QTLs) studies can reveal the adaptive genetic variation in a species (Lynch et al. 1999).

The findings of seed germination and seedling performance approaches of *C. sempervirens* (Chapter 3 and 4) could have resulted from inbreeding depression. This expectation resulted from the fact that our afforested populations are small and spatially scattered. The inbreeding depression is widespread in outcrossing species such as conifers and was found more frequently in isolated marginal populations (Mimura and Aitken 2007). In general, small populations are more prone to inbreeding depression which can lower fitness in their offspring (Kärkkäinen et al.1999; Rajora et al. 2002).

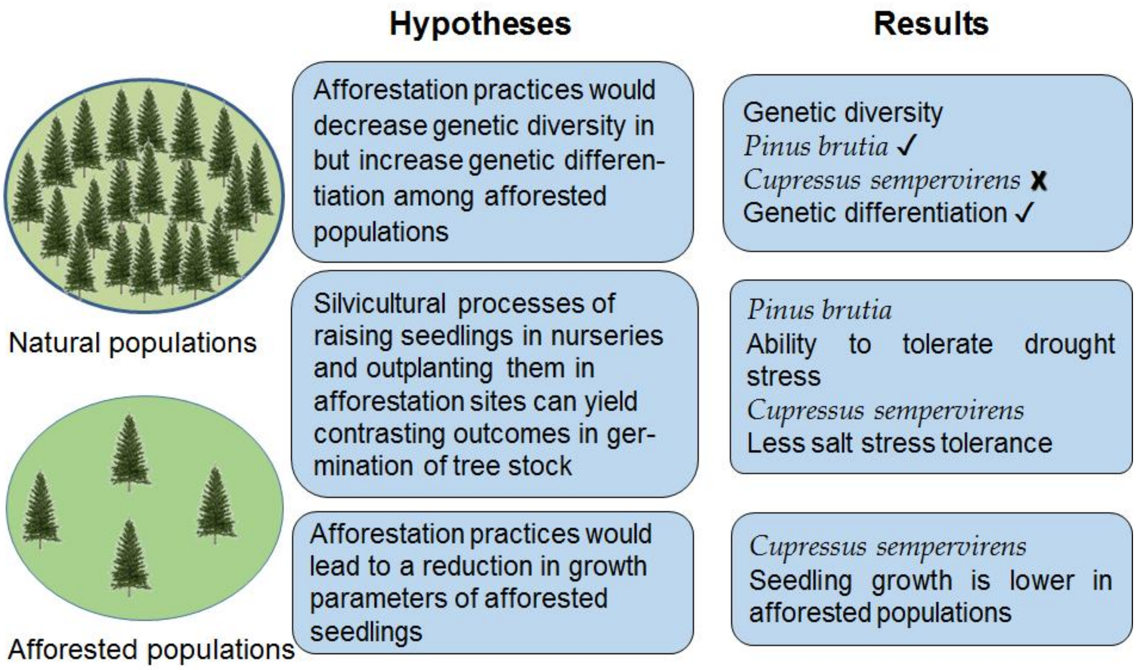

Fig. 5 Summary of the main hypotheses and results of the thesis. Trees here symbolize the population types. Natural populations are large and continuous; afforested populations are small and spatially isolated.

The environmental maternal conditions could also have been affected the performance of seeds and seedlings from the afforested *C. sempervirens* populations. In this regard, seeds and seedlings of *Pinus pinaster* from favourable maternal environment outperformed seeds and seedlings from stressful maternal environments (Cendán et al. 2013; Zas et al. 2013).

IMPLICATION FOR CONSERVATION

A high genetic diversity in afforested populations is important both for forestry and conservation. As the results of this thesis show, afforested populations of *P. brutia* are genetically less diverse than natural populations. Afforestation practice could account for this fact by sampling cones from a higher number of mother trees and source populations. It is recommended that at least ten to 25 seed trees as sufficient to represent the genetic diversity of a population (Eldridge et al. 1994; Williams et al. 2002). In this context, the selection of 25-34 mother trees (İçgen et al. 2006) and 100 mother trees (Stoehr and El-Kassaby 1997) resulted in the transfer of respective genetic diversity in natural populations to clonal seed orchards. However, genetic erosion could occur during seed and seedling production phase. Conversely, large populations of several thousand are required to have a high probability that multiple copies of low-frequency alleles are maintained (White et al. 2007). Furthermore, in order to maintain a high level of genetic diversity, the common practice of selecting the mother trees on the bias of phenotypic superiority should be alleviated. It might be supposed to state a grade range of favorable phenotypic traits and within each range, a specific number of mother trees should be selected. Nevertheless, small trees should not be considered as seed parents although it may be easier to collect seeds from them (Finkeldey and Hattermer 2007).

In breeding programs, in most cases, seeds are collected, extracted and stored as bulked seedlots comprises seeds of several parents with underdetermined proportions of each parent (El-Kassaby and Thomson 1990). If parental seedlots vary in their germination speed (which is often the case), the usual practice of thinning multiple seedlings (and leaving the largest ones) and culling could lead to a loss of genetic diversity. Consequently, the consecutive bottlenecks in the process of seed and seedling production in nurseries can be bypassed if practitioners would adopt single seed or individual family sowing in the nursery (El-Kassaby and Thomson 1996).

The sustainability of future afforestation can be enhanced by selecting the correct planting material, i.e. the genotypes best adapted to the given site conditions, selected from provenance trials of relatively long duration (Schiller and Atzmon 2009). Field trials are indispensable experiments for genetically efficient selection of populations suitable for the establishment of plantations and for the utilization of genetic variation within populations by breeding (Finkeldey and Hattermer 2007). In this context, if 25 mother trees are hard to find in small local population, it might be not eligible for entry into provenances experiment (Williams et al. 2002). Keeping single-tree seedlots separate until sowing is advisable and also necessary if the provenance test is to be combined with a progeny test (Finkeldey and Hattermer 2007). Furthermore, research in afforestation needs to consider maternal effects, defined as the direct contribution of the maternal environment to the offspring phenotype (Roach and Wulff 1987) when inspecting performance of early life stages.

In case that afforestation programs would rely on collecting seed material from older plantations such the case of our afforested populations. The afforestation programs would benefit from a catalogue of seed source material so that all reproductive materials are traceable back to source. Moreover, if afforestation is planned to be established in areas with hot dry climatic conditions, it might be suggested to expose the seedlings to some levels of drought and salt stress during nursery culture. Hence, the individual seedlings that will survive and grow well might be ready to tolerate the stressful environmental conditions at plantation sites. In addition, the conservation of the afforested *P. brutia* populations should be based on maintaining a substantial effective population size that could be promoted by replantation with local source (Diaz et al. 2001).

In conclusion, afforestation practice needs to be altered in order to preserve genetic diversity in afforested populations. Practitioners should collect seed materials from multiple individuals and multiple populations, include a wide variety of maternal phenotypes, plant seeds per individual or family and forego severe selection processes from seedling stock. Based on the results of this thesis, it might be suggested that for further afforestation programs in arid and semi-arid areas, selection of seed materials from afforested *P. brutia* populations in the corresponding area should be taken into account. For *C. sempervirens* and according to the thesis results, a systematic collection of seed materials from natural populations for future afforestation programs might be recommended.

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PPENDIX 1

PUBLICATIONS OF THE DISSERTATION

Batoul N. Al-Hawija, Viktoria Wagner and Isabell Hensen (2014). Genetic comparison between natural and planted populations of *Pinus brutia* and *Cupressus sempervirens* in Syria. *Turkish Journal of Agriculture and Forestry* 38: 267-280.

Batoul N. Al-Hawija, Susanne Lachmuth, Erik Welk and Isabell Hensen (2015). Performance of seedlings from natural and afforested populations of *Cupressus sempervirens* under different temperature and moisture regimes. *Plant Species Biology* 30(4): 257-271

Batoul N. Al-Hawija, Monika Partzsch, Viktoria Wagner and Isabell Hensen (2014). Germination differences between natural and afforested populations of *Pinus brutia* and *Cupressus sempervirens. Silva Fennica* 48(4): 1-18.

OTHER PUBLICATIONS BY THE AUTHOR

Batoul N. Al-Hawija, Monika Partzsch and Isabell Hensen (2012). Effects of temperature, salinity and cold stratification on seed germination in halophytes. *Nordic Journal of Botany* 30(5): 627-634.

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Halle (Saale), 15. September. 2014

APPENDIX 2

DECLARATION OF OWN CONTRIBUTIONS TO THE THESIS PUBLICATIONS

As several co-authors contributed to the thesis manuscripts, in the following the percentages of own work are shown.

First study (Chapter 2):

Genetic comparison between natural and planted populations of *Pinus brutia* and *Cupressus sempervirens* in Syria.

- Collection of plant material and field data: 95%
- RAPD analysis: 100%
- Statistical analysis: 95%
- Manuscript: 90%

Second study (Chapter 3):

Germination differences between natural and afforested population of *Pinus brutia* and *Cupressus sempervirens*.

- Collection of seed material and field data: 95%
- Germination test in the lab: 98%
- Statistical analysis: 80%
- Manuscript: 80%

Third study (Chapter 4):

Performance of seedlings from natural and afforested populations of *Cupressus sempervirens* under different temperature and moisture regimes.

- Collection of seed material and field data: 95%
- Growth experiment in the greenhouse: 100%
- Statistical analysis: 80%
- Manuscript: 80%

DECLARATION OF SELF-CONTAINED WORK / EIGENSTÄNDIGKEITSERKLÄRUNG

Hiermit erkläre ich, dass die vorliegende Arbeit von mir bisher weder der Naturwissenschaftlichen Fakultät I – Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde.

Ich erkläre, dass ich mich bisher noch nicht um einen Doktorgrad beworben habe.

Ferner erkläre ich, dass ich diese Arbeit selbständig und nur unter Zuhilfenahme der angegebenen Hilfsmittel und Literatur angefertigt habe. Wörtlich oder inhaltlich entnommenen Stellen aus den benutzen Werken sind als solche kenntlich gekennzeichnet.
Halle (Saale), den 15.09.2014

Unterschrift: ______________________________

(Batoul N. Al-Hawija)