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VARIATION IN RELATIVE GROWTH RATE IN THE CHENOPODIUM ALBUM AGGREGATE IN RELATION TO PLOIDY LEVEL.

DIPLOMA THESIS

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DIPLOMA THESIS TOPIC

Author of thesis:	Steven Musoni
Study programme:	Engineering Ecology
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Thesis supervisor:	prof. Mgr. Bohumil Mandák, Ph.D.
Supervising department:	Department of Ecology
Language of a thesis:	English

Thesis title: **Variation in relative growth rate in the Chenopodium album aggregate in relation to ploidy level**

Objectives of thesis:

Variation of relative growth rate (RGR) and other growth parameters in the annual diploid-polyploid complex of Chenopodium album aggregate will be examine in relation to ploidy level of individual species tested. Members of the C. album aggregate pose a difficult taxonomical challenge in that it is composed of many only weakly differentiated and morphologically overlapping entities with worldwide distribution. However, thanks to the fact that individual species within C. album aggregate are uniform in the ploidy level and have low genome size variation, it is possible to identify them and test the effect of increasing ploidy level and genome size on basic growth parameters in experimental conditions.
In this study, a detailed growth analysis will be conducted on about fifteen species of various ploidy levels of C. album aggregate. All plants will be grown under experimental condition and the following questions are addressed:
1) What is the extent and pattern of variation of RGR and its components within tested species?
2) Do species of lower ploidy levels or lower genome sizes show lower RGR than species with large genomes?

	3) Is RGR correlated with other parameters such as the size of distribution range, degree of invasiveness and other species specific ecological conditions?
Methodology:	We will use climatic chambers to determine relative growth rate of fifteen representatives of diploid-polyploid complex of Chenopodium album aggregate in strictly experimental conditions. RGR data will be analysed in relation to ploidy level and genome size.
The proposed extent of the thesis:	60 pages
Keywords:	Chenopodium, genome size, ploidy level, relative growth rate

Recommended information sources

1. Hendry G.A.F. and Grime J.P. (1993): Methods in Comparative Plant Ecology. A Laboratory Manual. Chapman & Hall, London.
2. Hunt R., Causton D. R., Shipley B nad Askew A. P. (2002) A modern tool for classical plant growth analysis. Annals of Botany 90: 485–488.
3. Krak K., Vít P., Belyayev A., Douda J., Hreusová L. & Mandák B. (2016): Allopolyploid origin of Chenopodium album s. str. (Chenopodiaceae): A molecular and cytogenetic insight. PLoS ONE 11(8): e0161063. doi:10.1371/journal.pone.0161063.
4. Mandák B., Krak K., Vít P., Pavlíková Z., Lomonosova M. N., Habibi F., Lei W., Jellen E. N. & Douda J. (2016): How genome size variation is linked with evolution within Chenopodium sensu lato. Perspectives in Plant Ecology, Evolution and Systematics 23: 18–32.
5. Vít P., Krak K., Trávníček P., Douda J., Lomonosova M. N. & Mandák B. (2016): Genome size stability across Eurasian Chenopodium species (Amaranthaceae). Botanical Journal of the Linnean Society 182: 637–649.

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DECLARATION

STUDENT'S DECLARATION

I STEVEN MUSONI, hereby declare that except for references to other people's work which have been duly cited and acknowledged, this action research is the result of my own effort and that it has neither in whole nor in part been presented elsewhere.

SIGNATURE……. DATE…….

(STEVEN MUSONI)

SUPERVISOR'S DECLARATION

I hereby certify that preparation and presentation of this thesis were supervised in accordance with the guidelines binding the supervision of diploma thesis laid down by the Czech University of Life Sciences.

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ABSTRAKT

Rod *Chenopodium* zahrnuje 100–170 druhů. Většina z nich se vyskytuje v temperátním pásu na nejrůznějších stanovištích. V rámci této skupiny rostlin se vyskytují jak druhy domestikované na různých místech světa, tak i velmi nepříjemné polní plevele. Mnoho z druhů náležejících do rodu *Chenopodium*má polyploidní původ, což jim částečně umožňuje přežívat na nejrůznějších, obvykle nepříhodných, stanovištích. Hlavním záměrem této studie bylo porovnat vybrané růstové parametry mezi osmi eurasijskými druhy rodu *Chenopodium*sbíraných na různých lokalitách v Řecku, České republice a Číně. Testovali jsme následující druhy *C.acuminatum*(2x), *C.album* (6x),*C.ficifollium*(2x), *C.karoi*(4x),*C.novopokrovskyanum*(4x), *C.opulifollium*(4x), *C.stratiforme*(4x)a *C.strictum*(4x). Po 14 dnech studené stratifikace, byly jednotlivé druhy přesunuty do růstové komory, v které byl nastaven standardní režim (22°C den trvající 14 hodin a 15°C noc trvající 10 hodin). Humidita byla nastavena na 70 %. Rostliny byly odebírány ve dvou destruktivních sklizních – třetí a pátý týden po vyklíčení semen. U všech byla změřena relativní růstová rychlost (RGR) a další veličiny jako Specific leaf area (SLA), Net AssimilationRate (NAR), LeafMass Ratio (LMR) a Leaf Area Ratio (LAR), které byly vypočítány pomocí analýzy suché biomasy z první a druhé sklizně. Překvapivě jsem nenašel žádný rozdíl v RGR mezi jednotlivými druhy merlíků a mezi druhy s různými ploidiemi. Nicméně jsem zjistil několik statisticky významných rozdílů v případě SLA, LAR a LMR. Taktéž jsem porovnával RGR ve vztahu k invazivnosti a fylogenetickému původu. Ani zde jsem však nenašel statisticky významné rozdíly.

Klíčová slova: *Chenopodium* ,velikost genomu, úroveň ploidy, relativní míra růstu

ABSTRACT

Genus *Chenopodium* is comprised of about 100-170 species which are distributed mainly in temperate zone. Some *Chenopodium* species are among the world's worst weeds. However, some species are domesticated for food and source of medicine in different parts of the world.

Most *Chenopodium* species are polyploids which is an important trait as far as evolution is concerned because when some species undergo polyploidization, it increases their adaptability in different habitats as their genome size is enlarged and can perform many functions that help the plant resisting in different habitats. The main purpose of this study was to compare relative growth rate among 8 Eurasian *Chenopodium* species from Greece, the Czech Republic and China with different ploidy levels (i.e. hexaploid, tetraploid and diploids). RGR experiment was carried out in a laboratory on 8 Eurasian *Chenopodium* species with different ploidy levels collected from different populations: *C.acuminatum*(2x), *C.album* (6x), *C.ficifollium*(2x), *C.karoi*(4x),*C.novopokrovskyanum*(4x), *C.opulifollium*(4x), *C.stratiforme*(*4x)* and *C.strictum*(4x). After 14 days of cold stratification, germinated *Chenopodium* species were introduced in a growing chamber and the regime was set as the ISP standard temperature was 22℃ by day and 15℃ by night, the ISP standard day had 14 hours and 10 hours day and night respectively. Humidity was set at 70%. Two destructive harvests were done in the third week and the fifth week in order to measure leaf area, leaf weight, stem weight and root weight. Finally, these parameters were used to measure RGR and its components. Specific leaf area (SLA), Net Assimilation Rate (NAR), Leaf Mass Ratio (LMR) and Leaf Area Ratio (LAR) were calculated using plant biomass from the first and second harvest. Surprisingly, there was no significant variation in RGR among *Chenopodium* species and among the ploidy levels. However, there was a significant variation in SLA, LAR and LMR among *Chenopodium* species. We also compared RGR in relation to plant invasiveness and phylogeny. However, there was no significant variation in RGR in relation to invasiveness and phylogeny.

Key words**:** *Chenopodium*, Genome size, Ploidy level, Relative growth rate

Table of contents:

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CHAPTER ONE

1. Introduction

Genus *Chenopodium* is a medium-large genus consisting of about 100-170 species that belong to Chenopodiaceae or Amaranthaceae family. The genus includes herbaceous and that are distributed almost everywhere in the world but most abundant in the temperate zone. Molecular data analysis has confirmed that *Chenopodium* species undergo polyploidization and hybridization, this increases their genome size and sterile hybrids which undergo polyploidization become biologically fit. (Singh 2010). Some species of genus *Chenopodium* are classified among the top five distributed plants in the world (i.e. *Chenopodium album* L), however, this species is cultivated in some places and exist as a weed in other places of the world (Williams 1963).

Growth rate and growth, in general, are most important features that show plant fitness. It is very common that plants have different growth rates even when they are in the same ecological condition or provided with the same requirements. Genetic and phenotypic factors can lead to variation in growth rates among species. Domesticated plant species with traits that increase their growth rates are preferred because they can strongly compete with invasive species and can resist during adverse ecological conditions. (Shipley 2000; Li et al.2016; Oguchi et al.2016).

RGR is a fundamental tool for understanding the species life history and strategies as it incorporates physiological, morphology and anatomy of a species (Grotkopp et al.2010). The major traits that determine RGR may be influenced by environmental conditions in a certain habitat (i.e. light availability and Carbon dioxide Concentration), this is because the major RGR components or RGR determinants depend on specific traits. (Li et al.2016).

To assess if the variation in RGR among species is caused by inherent factors, RGR experiment is carried out in optimum and controlled conditions (i.e. all the species are given uniform growing conditions; South 1995).

1.1. Aims and objectives of the study.

The main aim of the present study is to assess the variation of RGR in Eurasian *Chenopodium* species in relation to ploidy levels.

Objectives

The present study consists of two main parts, the first part is the review of the existing works and research about genus *Chenopodium* especially the Eurasian species that will be targeted in this study and also reviewing the previous works on RGR. The second part will be carrying out a Relative growth experiment to investigate if there are any variations in RGR among *Chenopodium* species with different ploidy levels and to compare the variation of RGR among *Chenopodium* species with different ploidy levels.

Our main aim will be reached by conducting an experiment where seeds from different species of *Chenopodium* will be planted in a growing chamber. The variation of RGR will be assessed by measuring RGR and its components using stem weight, leaf area, leaf weight and root weight.

The subject of the study is Eurasian *Chenopodium* species with different ploidy levels (i.e. 2n=2x=18, 2n=4x=36 and 2n=6x=54) from different localities in three countries (Czech Republic, Greece and China).

The hypotheses were that there will be variation in RGR among *Chenopodium* species, there will be also variation in RGR among ploidy levels (i.e. hexaploid, diploid, and tetraploid), species with higher ploidy levels will express higher RGR and invasive species will express a higher RGR than non-invasive species.

Many experiments have been done about the genus *Chenopodium* but there is no much works have been done to investigate the variation of RGR in *Chenopodium* species that differ by genome size. Studying genus *Chenopodium* is very important in many ways for example implications about management and control of the *Chenopodium* weed species and on the other hand, *Chenopodium* species of a great importance are known so that they can be protected and conserved.

The main aim of this diploma thesis is basically assessing the variation in RGR in *Chenopodium* species in relation to ploidy levels.

Research questions:

1) What is the extent and pattern of variation of RGR and its components within tested species?
2) Do species of lower ploidy levels or lower genome sizes show lower RGR than species with large genomes?
3) Is RGR correlated with other parameters such as the size of distribution range, the degree of invasiveness and other species-specific ecological conditions?

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Ecology and biology of Chenopodium species

Genus *Chenopodium* is a medium-large genus comprising of 100-170 morphologically, genetically and phenotypically different species that are distributed almost in all parts of the world especially the temperate zone although the exact number of species in the genus vary among authors. (Bhargava et al. 2005; Bhargava et al. 2007; Bhargava et al. 2010; Fuentes-Bazanet al. 2012). *Chenopodium* species are both perennial and annual plants (Bargava et al. 2007).

Some *Chenopodium* species are economically important while others are ranked as the world's worst weeds. (Bargava et al. 2007) while other species exist both as wild and cultivated at the same time (Kolano 2007; Bargava et al. 2007).

The genus is also comprised of diploid, polyploid, native *Chenopodium* species to some areas. *Chenopodium* species have a great degree of polyploidization that varies from triploids species to decaploids. Tetraploid species are the common *Chenopodium* species while triploids rarely exist. (Bhargava et al.2007; Davidson et al.2011; Walsh et al. 2015). *Chenopodium* species rarely exist on their own, some species colonise unoccupied places and others are found with other crops where they are weeds.

Polyploid *Chenopodium* species have traits (i.e. a high degree of plasticity) which make them successful weeds hence outcompete cultivated crops and become noxious agricultural weeds. (Walsh et al.2015).

According to Bhargava et al. (2005), *Chenopodium album* is a harmful weed to another economically important *Chenopodium* species *Chenopodium quinoa* wild that is domesticated and cultivated in India, however, there is no recommended herbicide to fight against this weed instead the weeds are removed by farmers using their hands which is a good method hence it has no effect on the target *Chenopodium quinoa* Willdenow plants.

Other *Chenopodium* species are among the worst weeds in the world and are some of the most widespread synanthropic plants on earth. This is the case of *C.album* complex

(Mandák et al.2012), and it is a hazard to corn farmers because it was shown that it resists to the commonly used herbicides like Dicamba (3,6-Dichloro-2-methoxy benzoic acid) and Atrazine (1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) (Rahman et al.2014). Apart from *C.album*, they are many other species which are both resistant and tolerant to different kinds of herbicides (i.e. *C. strictum* is tolerant to Atrazine and Pyrazon). It is very important to know species that are resistant and those which can be killed by herbicides so that farmers can be able to know the appropriate method of eradicating weeds. (Solymosi et al.1986).

Invasive *Chenopodium* species have a higher degree of phenotypic plasticity which can also be another factor that may influence their morphology, physiological and fitness of a plant. Knowing that invasive species have a high phenotypic plasticity than native species can help farmers preventing them because once they colonise a new environment because they have high chances of outcompeting the native species due to their greater plasticity. Their great degree of plasticity can also help them to be distributed around the world because they can easily cope up with harsh environmental conditions. (Davidson et al.2011).

Due to taxonomical confusion and origin of some *Chenopodium* species, most of the works about *Chenopodium* are mainly on taxonomy and origin of genetic variation in *Chenopodium* species. They are few studies on economic, ecology and medicinal roles of *Chenopodium.* Only nutritional and medicinal roles are published but there might be other economic importance of *Chenopodium.* (Walsh et al. 2015).

2.1.2. Medicinal and nutritional value of *Chenopodium* species

Despite the fact that a large number of *Chenopodium* species are harmful agricultural weeds, a few *Chenopodium* species have nutritional values and economical use. (Bhargava et al.2005; Mandák et al. 2012; Bhargava et al. 2017). *Chenopodium* species serve as medicine and food for both humans and domestic animals like cattle, pig and birds. (Williams 1963; Dembitsky et al, 2008).

Only a small number of species is domesticated due to their nutritional and other economic values. However, the biggest number is wild and they can serve as medicine and as food for wild animals. (Bhargava et al.2007)

Researchers have only discovered nutritional and medicinal uses of *Chenopodium* species but much is still unknown (i.e. the origin of some polyploid species, the taxonomy of *Chenopodium* species, the genetic relationship among different species in the genus, the relationship between domesticated and wild species).(Atul et al. 2007)

The species that serve as grain crops in Andean region of South America are *C.quinoa*.wild, *C.berlandieri* subsp.nuffalliane(Stafford H.D.Wilson et Heiser) or Himalayan region of India (*C.giganteum* D.Don, C, *C.album L)* while *C.ambrosiodes L, C.botrys* L and *C.murale* L have medicinal values in different parts of the world. However, some species can play two roles at the same time whereby some parts can be used as medicine and other parts as food. *Chenopodium* species can be used both as traditional medicine as well as raw materials for pharmaceutical industries in manufacturing modern medicine (i.e. some species can be used to manufacture anti-itch drugs, painkillers, anthelmintic, antibacterial and antifungal to mention but a few). Stems, leaves and roots are the main parts that are mainly used for medicinal uses and are used to heal different diseases and disorders around the world. These parts are rich in chemicals like flavanols, ascaridole and monoterpenes which are used to make drugs. (Bhargava et al.2009).

2.1.3. Taxonomy of genus *Chenopodium*

Researchers have been struggling with the complex taxonomy of this genus for a long period of time and one of the causes of complications is extensive hybridization. (Walsh et al. 2015). Different studies have been made on genus *Chenopodium* and specific species under the genus but until today knowledge about *Chenopodium* especially its taxonomy is still not complete. The well-known example of taxonomic confusion can be seen in European *Chenopodium* species *Chenopodium album* and its relatives, for example, *C.album*, *C.opolifolium*, *C.strictum*, *C.suecicum* and *C.virgatum* which are often grouped as a single species aggregate. The cause of this confusion is said to be genetic and environmental factors. (Ranjbar 1995).

Almost all studies made about *Chenopodium* suggest that it is difficult to taxonomically classify all the species into their respective taxa. The factors that contribute to this conundrum are large number of species in the genus, polyploidization, hybridization, morphological differences, phenotypic plasticity, differences in genome size among

species and biogeographic diversity, however, the leading factor to the puzzle is differences in genomic constitution. Some species are morphologically similar and others are different which also causes confusion in intra and interspecific variations in the genus.

Many taxonomists and researchers have been trying for a long period of time to figure out the taxonomy puzzle of the genus, however, it is still poorly understood because some species tend to be morphologically similar but genotypically different which makes it difficult for the taxonomists to place them in the same taxon. (Cole 1961; Ohri 2015).

Not only classifying *Chenopodium* species is difficult but also finding the relationship between species is another confusing process but different researchers have used some characteristics for example grain characters, morphological character and chemical content in trying to figure out the relationship between different species of *Chenopodium* and they revealed that species that shared the flavonoid profile are related taxonomically, however, it is still a great confusion to botanists who try to classify *Chenopodium* species in their respective taxa (Rahiminejad and Gornall 2004; Atul et al. 2007).

Some groups of *Chenopodium* are difficult to be classified taxonomically because of frequent hybridization, self-fertilization, high degree of phenotypic plasticity, variable morphology and different ploidy levels. (Mandák 2012; Vít et al.2016)

According to Rahiminejad and Gornall (2004), taxonomical difficulties increase with the increase in ploidy levels. *Chenopodium* species are said to produce chemicals which can be used to identify the relationships among species based on the content of the chemicals like flavonoids, species can be grouped depending on chemicals they produce, however, hexaploid species can be morphologically similar and have the same chemical content which increases their taxonomical complexes but diploid and tetraploid species produced different chemical composition which can help in their taxonomic classification.

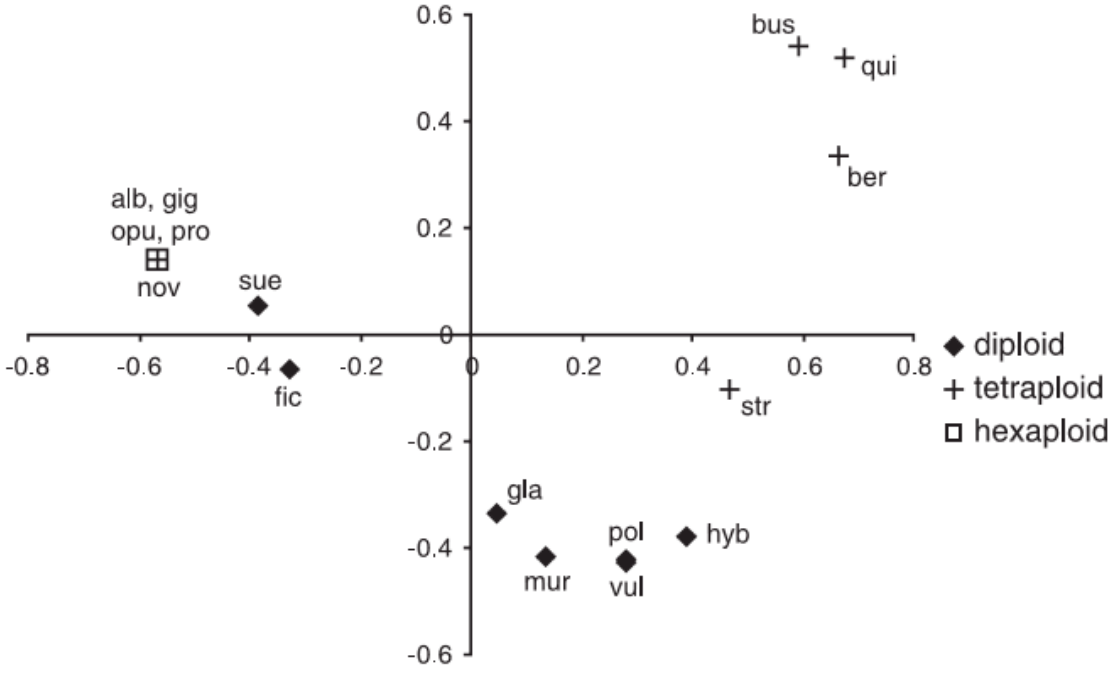

Fig.1. Sixteen *Chenopodium* species grouped according to their flavoid content, species' names are abbreviated by the first three letters.(i.e. alb-*C.album,* gig-*C.giganteum,* opu-*C.opulifolium,* pro-*C.probstii,*nov-*C.novopokrovskyanum,* sue-*C.suecicum,* fic-*C.ficifolium,* mur-*C.murale,* gla-*C.glaucum,* pol-*C.polyspermum,* hyb-*C.hybridum,* str-*C.strictum,* bus-*C.bushianum,* ber-*C.berlandieri,* qui-*C.quinoa).* (Rahiminejad and Gornall 2004).

Chenopodium species are distributed almost everywhere in the world. Weed species exist in cultivated fields, abandoned farmland, roadsides and along riverbanks. (Wang et al.2014). Eurasian *Chenopodium* species have been introduced to other places as crops. (i.e. *Chenopodium ficifolium* was introduced in North America). However, *Chenopodium* species that are native to southeastern Asia occur as weeds. (Mosyakin 2016). The biggest percentage of *Chenopodium* species are wild and uncultivated though some species may be cultivated and domesticated for a certain purpose. However, there are no studies about the relationship between domesticated *Chenopodium* and their wild ancestors. (Fuentes-Bazan et al.2012; Walsh et al.2015).

Chenopodium species are both annual and perennial plants that can grow in harsh environmental conditions because they can easily adapt and tolerate adverse environmental conditions like salty conditions. (Fuentes-Bazan et al.2012). Though some species can

tolerate and survive in extreme environmental conditions, their relative growth rate and yield increase when they are in their favourable conditions. (Williams 1963).

According to Davidson et al. (2011), the high degree of phenotypic plasticity can enable most of the species to survive in unusual ecological conditions (i.e. drought, soil, PH, pathogens, pests and diseases to mention but a few).

Chenopodium album is a weedy annual plant belongs to the genus *Chenopodium* and it is also cultivated in some places and consumed as food. It grows in all soils rich in Nitrogen, especially on wastelands. The leaves are un-wettable, mealy in appearance, can be used as a leaf vegetable and it possesses high nutritious value. (Bhargava et al.2005; Sighi 2010; Yerka et al.2012; Ravindhranath et al.2016)

Fig. 2: *Chenopodium album* plant, one of the Eurasia *Chenopodium* weeds species. (Ravindhranath et al.2016).

Chenopodium album can be found almost everywhere in the world and it cannot be affected by geographical climatic changes (i.e. it can exist and survive in unfavourable ecological conditions). (Williams 1963; Singhi 2010). It was reported as a major problem faced by

maize growers in New Zealand and was discovered to be the first herbicide-resistant weed in New Zealand. Atrazine is the herbicide used by maize growers but unfortunately, the weed is resistant to this herbicide. (Singhi 2010; Rahman et al.2014). This species is also a weed on *Chenopodium quinoa* wild grown in India, however, farmers use hands to remove the weed from the plantation. *Chenopodium album* is used as leafy vegetables in some parts of the world. (Gangopadhyay et al. 2002; Bhargava et al. 2005).

Chenopodium album is both semi-cultivated and wild. Cytologically, it exists as a diploid, tetraploid and hexaploid. The three cytological races of this species differ in their habitats and morphology. (Mehra and Malik, 1963; Kolano et al,.2007; Singhi, 2010; Rahman et al,. 2014).

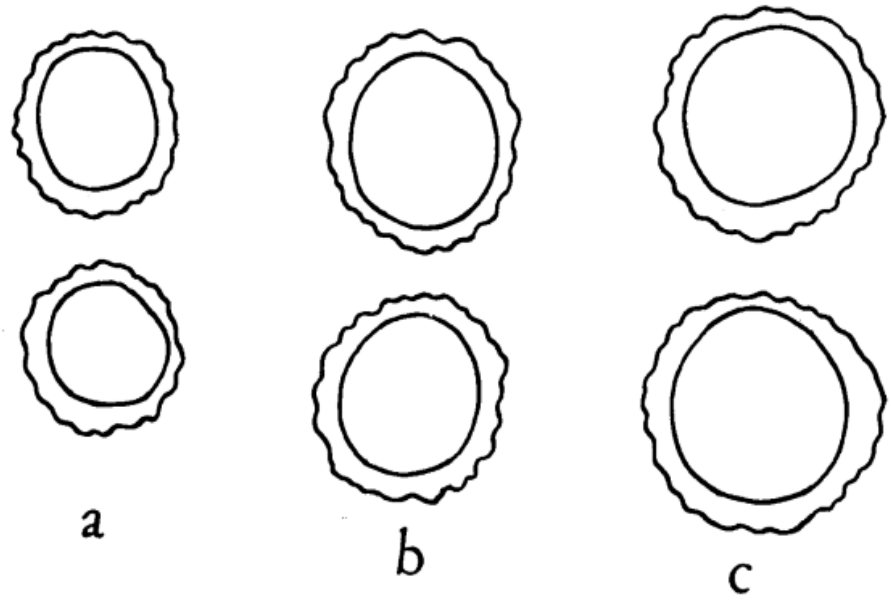

Fig. 3: The sizes of pollen grains of 3 different cytological races of *Chenopodium album*, a(2x), b(4x), c(6x). (Mehra and Malik 1963).

Another weed *Chenopodium* species in the present study is *C. acuminatum* though it is not harmful to any domesticated crops because it is found in abandoned areas hence does not harm any crop. It also has a high degree of phenotypic plasticity and it can be found in extreme habitats like sandy dunes. (Huang et al. 2013; Wang et al. 2014).

2.1.5. Stress tolerant *Chenopodium* species

A very important feature possessed by most of *Chenopodium* species (i.e. *Chenopodium quinoa, Chenopodium album, Chenopodium acuminatum* and *Chenopodium strictum*, to mention but a few) is having a high tolerance degree (i.e. tolerate harsh environmental conditions like low temperatures, salinity and drought). Apart from being resistant and tolerant to adverse environmental conditions, *Chenopodium* species can resist some pathogens and pests that might suppress their growth and reduce the yield of economically important *Chenopodium* species. (i.e. *Chenopodium quinoa* wild is resistant to some viruses and also produce some chemicals that protect it from being eaten by pests.) (Bhargava et al. 2005), however, some studies indicate that there are some pathogens which may negatively affect the yield and nutritive value of *Chenopodium* quinoa.(Jiri et al.2015).

Chenopodium album deals with some stress environment conditions by producing different types of seeds on the same plant individual which increases the rate of tolerance during seed germination as a paramount phase of plant growth. (Tanveer and Shah 2017).

Other *Chenopodium* species are halophytes but it is still difficult to tolerate saline environment during certain stages of growth and development of a plant, this study shows that *Chenopodium quinoa* which was introduced into a saline environment had a lower germination rate compared to the individuals that originated in the saline area, this implies that though *Chenopodium* species can be tolerant, however, it depends on whether individuals are native to a certain environment or they were introduced, it also depends on a phase of growth (i.e. germination where the degree of stress tolerance is low, Adolf et al. 2013).

According to Ramírez-Valiente (2017), some stress tolerant plants may have a low relative growth rate though they are no evidence to support this phenomenon. Despite the fact that many authors suggest that many *Chenopodium* species are tolerant to stressful conditions, *Chenopodium quinoa* that was introduced to central Europe is easily attacked by diseases caused by germs and thus reduce their nutritive value because they are not able to resist pests and pathogens (Shipley 2002).

2.2. Polyploidization and Hybridization

Polyploidization is a process whereby the entire genetic materials replicate to produce another copy of the genome. (Ramsey and Ramsey 2014). Polyploidy can be inherited. (Comai 2005). Polyploidy is a state of having more than two sets of Chromosomes (i.e.triploid, tetraploid, hexaploid, to mention but a few). The most common ploidy level is tetraploid and this condition is common in plants but uncommon in animals. (Comai 2005; Münzbergová 2007). Variation in genome size among plant species is a common phenomenon though it can be caused by other factors apart from polyploidization (i.e. punctual insertion/ deletion, irregular recombination and losses of the whole chromosome). (Mandák et al. 2016; Vít et al. 2016).

Polyploid domesticated plants rely on vegetative reproduction which makes their reproduction easy and sometimes can increase the growth rate. (Alix et al.2017). Polyploidization is related to hybridization because most of the hybrids are polyploids, both processes help in evolution as chromosomes are renewed from one generation to another. Polyploids have a high adaptation power and they can occupy different ecological habitats than their diploid ancestors. (Comai 2005; Mandák 2012; Tamayo-Ordóñez et al.2016; Mandák et al. 2016).

Polyploidization can be either autopolyploid (i.e. formed when a single genome becomes duplicated) or allopolyploidy (i.e. formed when two or more genomes are combined by hybridization) (Tamayo-Ordóñez et al.2016). Polyploidization is a very important genetic process that plays a role in the gradual change of phenotypic and genotypic traits of a living organism. It gives rise to species which are genetically and phenotypically different from their ancestors. Polyploidization is an evolutionary process in many plants as it involves gradual changes of genome size from one generation to the other (i.e. hexaploids offsprings can be produced by diploid progenitors). (Adams and Wendel 2005; Münzbergová 2007; Alix et al. 2017). However, the difference between the genome size of polyploidy and diploids is lower than the expected but becoming and remaining polyploid changes the structure and functioning of the organisms both at the genotype level and phenotype level because polyploidy leads to increase of genetic materials in the nucleus that is responsible for all the cell activities and the entire organism in general. (Comai, 2005; Kolano et al, 2007).

Different researchers considered polyploidization as a dominant factor for phenotypic variations among polyploids and these species were recognised by their physical appearance instead of their genetic constitution. (Ramsey and Ramsey 2014). Alix et al. (2017) suggested that polyploidization does not only increase the genetic materials but also lead to an increase in the size of some plant parts like leaves, stem and fruits which is another advantage of polyploidization in domesticated crops. Genome size variation can be either increase or decrease of genetic materials but all changes in genome size contribute to the evolution of plant species. (Mandák et al. 2016).

About 50% of higher plants have undergone polyploidization at some point during evolution and there is a relationship between polyploidization and crop domestication because the percentage of polyploidy in domesticated crops is higher than their wild counterparts. (Alix et al.2017). There is a high rate of polyploidy formation in flowering plants and 80% of them are polyploids, this changes their physical structure thus allowing them to colonise different habitats. (Comai 2005; Kolano et al. 2007; Černa and Münzbergová 2013; Alix et al.2017). Polyploidy induction can be applied to economically important plants like medicinal and ornamental plants to improve their traits hence adapting to adverse ecological conditions but it can also cause negative effects like infertility, reduction of plant height and production of watery fruits. (Tamayo-Ordóñez et al.2016). Polyploidy species are considered to have a higher seed production than their diploid ancestors which is another economic advantage of polyploidy species. (Münzbergová 2007)

Despite the fact that many studies have been made on the variation of genome size in plants, there is a lot of things that are still unknown. (Walsh et al. 2015). However, researchers are increasing their interest in studying polyploidization and many methods that are used to study the mechanisms of the process have been discovered. (Krak et al. 2016). Polyploidy detection can be done by counting the number of chromosomes during different phases of cell division (i.e. Metaphase, Alix et al. 2017)

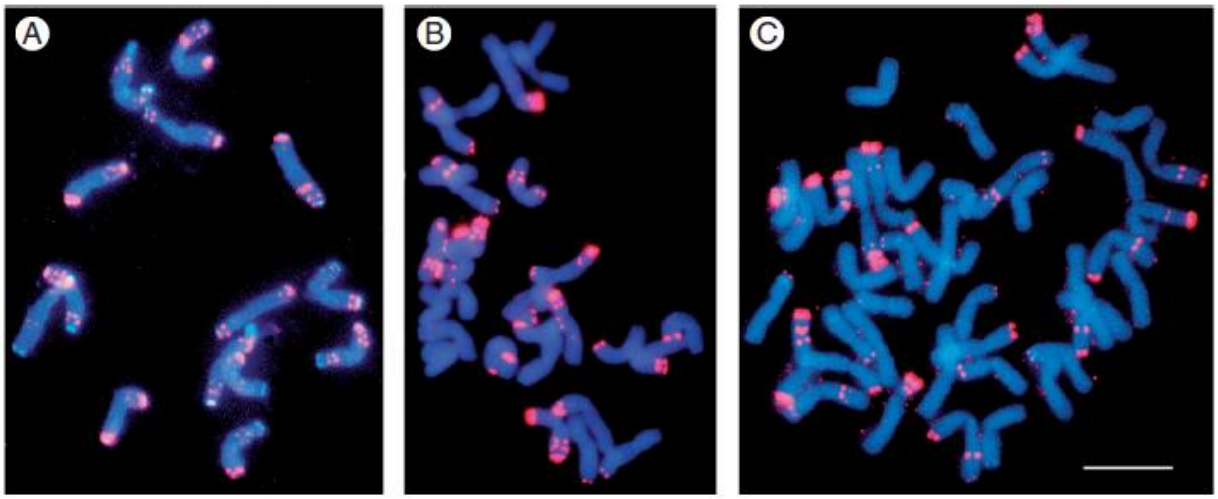

Fig.4: Chromosomes in metaphase of diploid (A), tetraploid (B) and hexaploid (C) wheat. (Alix et al. 2017).

Hybridization is also important as far as evolution is concerned because from one generation to another, hybridization leads to many changes in the genetic materials of species. These changes in genetic materials may create species with good phenotypes which can resist during climatic changes (i.e. interspecific hybridization will produce new species which have both characteristics from different parents thus increasing its adaptability). (Krak et al. 2016).

2.2.1. Polyploidization and hybridization in *Chenopodium* species

More than 70% of *Chenopodium* species are polyploids, however, the origin of some polyploid species is not well understood and is unclear (i.e. the origin of hexaploid *Chenopodium album).*(Comai 2005; Kolano et al. 2007; Walsh et al. 2015) and several polyploid species in this genus are economically important for example *Chenopodium quinoa* wild and some have been domesticated while other polyploidy species are among the world's worst weeds (i.e. *Chenopodium album*). (Walsh et al.2015; Krak et al. 2016).

Generally, *Chenopodium* species (i.e. both polyploidy and diploid) are considered to be agricultural weeds, however, some polyploids have been domesticated at a higher rate compared to their diploid counterparts. (Walsh et al. 2015).

Genetically and epigenetically, polyploid *Chenopodium* species are more advantageous than their diploid relatives because after polyploidization, there is enlargement of the

nucleus which controls all the cell activities thus allowing more convoluted processes to take place hence adjusting the physiological and morphological structures of the organism. (Comai 2005; Alix et al. 2017).

Like in many plants, polyploidization in genus *Chenopodium* is a paramount evolutionary process and it is brought up by hybridization, the most economically important *Chenopodium* species are polyploids and they are formed as a result of hybridization.(Fuentes-Bazan et al. 2012). The existence of varieties in genome size in *Chenopodium* makes this genus suitable for researchers who carry out comparative studies based on ploidy levels and genome size in general. (Mandák et al.2016).

Polyploidization can naturally happen as for the case of *Chenopodium* species but it can also be artificially induced to improve the traits of the plants as polyploid plants have high-quality traits than their diploid ancestors. (Van Laere et al. 2010). Genome size variation among species can be at the genus and family level (interspecific genome variation) or at the species level (intraspecific genome variation) for instance some *Chenopodium* species have different ploidy levels at the same time (i.e. *Chenopodium album* has diploid (2n=2x=18), tetraploid 2n=4x=36 and hexaploid 2n=6x=54). However, the most reported ploidy level in *C.album* is hexaploid. (Williams 1963; Ranjbar 1995; Kolano et al.2007; Vít et al.2017).

Polyploidy species are able to occupy vast geographical ranges and ecosystems compared to their diploid ancestors because polyploidization increases the level phenotypic plasticity. (Ramsey and Ramsey 2014; Sánchez Vilas and Pannell 2017).

Genome size variation is common and very essential in flowering plants, the range can even be bigger within the members of the same species. All the positive impacts of genome variation are not known, however, genome size causes strange phenotypic characteristics which can affect the growth and development of plants positively. (Mandák et al.2016).

Generally, there is a variation in genome size among species and within species but *Chenopodium* species show a stable genome size which can be used for their taxonomical classification. (Vít et al. 2016).

Polyploidization and hybridization are processes which help *Chenopodium* species to give rise to many other species with different genome size mostly polyploid species. *Chenopodium album* can hybridize with different *Chenopodium* species to give rise to many *Chenopodium* species both diploids and polyploids though hybridization also contributes to the taxonomic conundrum of the genus. (Mandák et al. 2012).

Polyploidization found in *Chenopodium* species is linked with hybridization process which also has an influence on the morphology of *Chenopodium* species hence advantageous to *Chenopodium* species because hybrids are morphologically different which enable them to be more resistant to adverse environmental conditions compared to their parents. (Fuentes-Bazanet al.2012). However, some domesticated *Chenopodium* species can also be morphologically differentiated according to the structure of their flowers. (Bhargava et al.2005).

Frequent hybridization and morphological diversity in polyploid *Chenopodium* species are linked phenomena and each has a positive correlation with each other. (Ohri 2015). Polyploidization found in *Chenopodium* species is linked with hybridization process which also has an influence on the morphology of *Chenopodium* species hence advantageous to *Chenopodium* species because hybrids are morphologically different which enable them to be more resistant to adverse environmental conditions compared to their parents. (Fuentes-Bazan et al.2012). However, some domesticated *Chenopodium* species can also be morphologically differentiated according to the structure of the flower. (Bhargava et al. 2005).

Hybridization is also an important evolutionary process common in plants and increases genetic diversity hence help organisms to increase their ability to tolerate stress though sometimes offsprings which are formed as a result of hybridization are not fertile. (Xi et al, .2017).

Many *Chenopodium* polyploids are formed as a result of hybridization (i.e. allohexaploid *Chenopodium album* originated from diploid *Chenopodium focifolium* and tetraploid *Chenopodium strictum*. The genome size of a species can be used to determine the type of hybridization, however, hybridization between members of *Chenopodium* is not a common phenomenon. *(*Vít et al. 2016).

The genus is characterized by small, invisible and often densely clustered flowers that are pollinated by wind hence it is difficult for isolated species to cross-breed though crossbreeding can take place at a small level. (Ranjbar 1995). Species of genus *Chenopodium* are generally self-compatible where artificial hybridization is difficult because normal emasculation and cross-pollination cannot take place. (Wilson 1980).

Polyploidization and hybridization can lead to the production of new *Chenopodium* species both diploids and polyploids, for example, polyploid *Chenopodium quinoa* hybridizing with diploid *Chenopodium album* to produce a new hexaploid individual species (Ohri 2015).

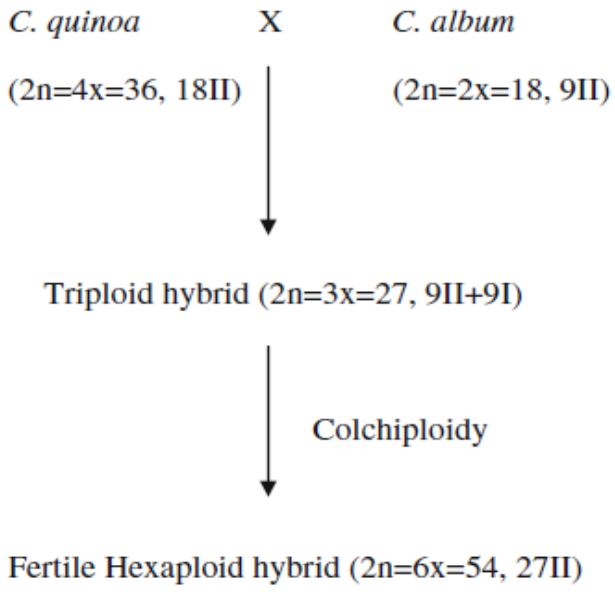

Fig.5: Cross between *Chenopodium quinoa* and *Chenopodium album.*

2.2.2. Why studying *Chenopodium*?

Despite the fact that most *Chenopodium* species are wild and harmful agricultural weeds in many parts of the world, there are a few important *Chenopodium* species which are even more important than some commonly cultivated crops (i.e. *Chenopodium quinoa* serves as grain and as medicine, *Chenopodium pallidicaule* and *Chenopodium berlandieri* subsp. nuttalliae are grain and vegetables and there are many wild *Chenopodium* species can serve both as medicine and as food for both domestic and wild animals in different parts of the world (Atul et al. 2007).

When comparing the content of minerals between *Chenopodium quinoa* and other common crops, *C.quinoa* shows a high content of minerals than common plants including beans, wheat and barley.(Bhargava et al. 2005). Some of these species are sources of nutrients especially in the villages of developing countries because they are consumed as leafy vegetables. They grow fast and tolerate unusual environmental conditions (Bhargava et al.2010).

Chenopodium species are not just source of food to human and animals but they are also major source of medicine as the biggest percentage of the world's population depends on traditional medicine according to World Health Organisation (WHO). These species are sources of more than 300 chemical compounds which are used to make modern medicine that cure many diseases and disorders (Gohar et al.2002; Kakonova-Nedialkova et al.2009).

The leaves, stems and roots of *Chenopodium* species have been used in many places of the world to heal different diseases and disorders because *Chenopodium* species are rich in chemicals that can be used in making drugs (i.e. flavanols, ascaridole, monoterpenes and monoterpenes to mention but a few), all these chemicals can be extracted from different *Chenopodium* species and can be used to heal a multitude of disorders like digestive disorders, respiratory disorders, circulatory disorders and nervous disorders to mention but a few. (Dembitsky et al. 2008)

2.3. Growth rate.

Growth is an increase in dry mass, volume, length and area due to cell division mainly mitosis which play a role in the multiplication of somatic cells. Plant growth analysis enables us to have a deeper understanding and explicit predictions for a wide range of plant morphological and physiological factors that may hinder or favour plant growth rate. (Tessmer et al. 2013).

Plant growth analysis helps us to understand the nature and ecology of a plant hence important

in agronomy because it can reveal the basic needs of some plant species for effective growth to take place. (Hunt et al. 2002)

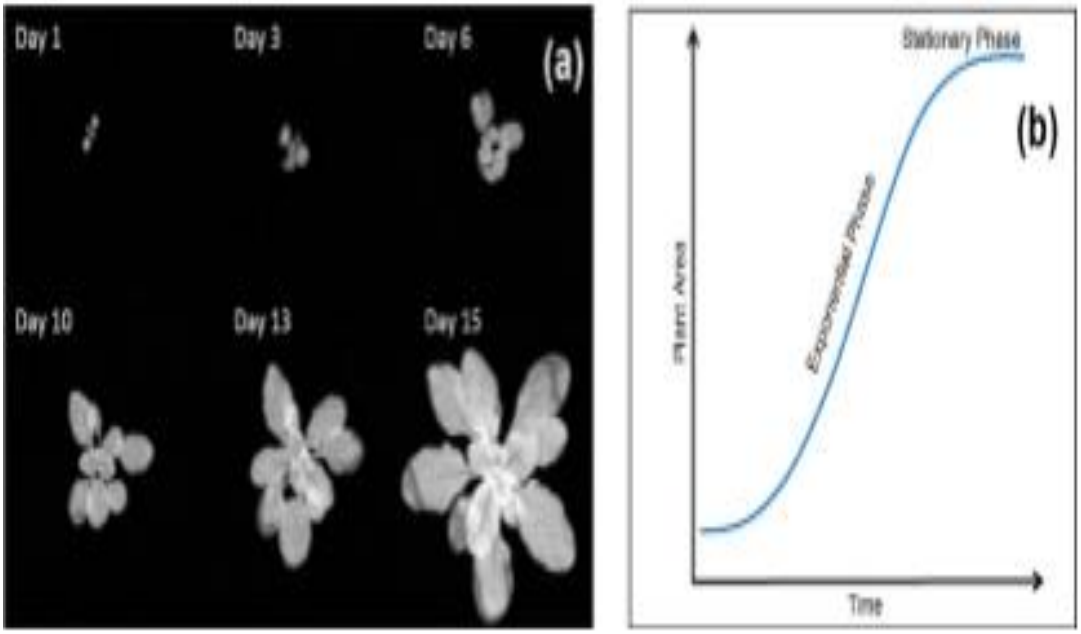

Figure 6: An example of plant growth and idealised general curve of typical higher plants. Images of *Arabidopsis* wild growing from day 1 to day 15. (Tessmer et al. 2013).

An idealised growth curve of higher plants means growth is directly proportional to time (i.e. plant height, mass and volume increase with time). (Tessmer et al. 2013). Plant growth rate and time of its cycle are primarily affected by environment and climatic conditions (i.e. if the environmental conditions are optimal, the rate of growth rate will be high and the cycle will be shorter but when the climate and environmental conditions are unfavourable, growth rate will be retarded and the cycle will be very long). (Koca and Erekul 2016)

Plant growth rate is determined by either biotic or abiotic factors. (Kabay et al. 2017).Generally, plant growth has two major components; absolute growth rate (AGR) and Relative growth rate (RGR). The common thing about the two components is that we calculate them by measuring the biomass at a given time during the growth of a plant. We can also make inferences about physiological activity and investigating the causes of variations in growth rates of the same plants grown in the same conditions such as fertilizers, temperature, humidity, light and Carbon dioxide concentration though we can

assess the variations in growth rates of plants grown in different conditions and plants which are genetically different. Assessment of growth rates is better achieved when comparing different species which are at the same age though their biomass might be different. This is done either when the study plants are planted at the same time or by measuring their growth rate parameters when they have the same age. (Li et al. 2016).

Plant growth analysis is very important in assessing the role of different physiological stages during both generative and vegetative periods because of the simple primary data taken in form of weight, area and volume. All the plants' vegetative parts help to investigate all the process taking place in the entire plant as growing and development is taking place. (Koca and Erekul 2016)

Growth rates in plants are determined by both biotic and abiotic factors available (i.e. light, temperature, nutrients and Carbon dioxide concentration), the amount of resources captured and the productivity of the used resources. Vegetative parts of the plants especially the leaves are of a great importance in plant productivity due to their role in carbon (C) assimilation by means of photosynthesis and transpiration. Therefore, leaf morphological traits determine the resource uptake and resource use efficiency and thus, leaf traits play a big role in determining plant growth rates. (Li et al. 2016)

Growth analysis is very important as it answers some question, for example, *Which factors cause fast-growing species to grow faster than slow-growing species?, How important are the variation between the rate of photosynthesis and respiration in biomass allocation in both morphology and chemical composition of a plant species?*, using RGR as a method of growth analysis will help us to get those questions answered as its components for instance NAR and LAR deals with physiological and morphological processes taking place in plant. (Poorter and Remkes 1990)

Other leaf traits like leaf lifespan, specific leaf area (SLA), rates of photosynthesis, rates of respiration and concentration of Phosphorous and Nitrogen can also play an important role in plant adaptation and hence alter growth rates in different ecosystems and different growing conditions. (Ramírez-Valeinte et al.2017).

Li et al.(2017) suggests that morphological and physiological characteristics of a leaf can be used in explaining the variation of growth rates among plant species however, the entire

crown size and structure can also be used in estimating the differences in growth rates as it determines the resources (i.e. light and carbon dioxide) captured by a plant.

2.3.1. RELATIVE GROWTH RATE (RGR).

RGR can be defined as a systematic measure of growth with an advantage of paying no attention to the innate differences between different species so that their performances can be compared equally. RGR is used to indicate how plants use the products of photosynthesis in growth and development. Many researchers have developed an interest in studying RGR and factors that may affect it, however, most studies are carried out under controlled conditions (i.e. greenhouse, growing chambers and laboratories).(Puglieli et al.2017).

RGR is a convoluted phenomenon that can be determined by a myriad of factors (i.e. physiology, ploidy levels, the morphology of vegetative parts, biomass partitioning, elevation and environmental. All these factors determine the variations of RGR components which directly influence RGR. Plants of different species can differ in their RGR and this is due to differences in their genetic makeup hence contributing to the variation in RGR. It is obvious that plants that are grown in fertile soil express a high RGR than plants grown in nutrient-poor soils. (Poorter et al.2005; Puglieli et al.2017).

For any plant species or individual to acquire maximum RGR, all the entire plant parts must actively be involved in all the physiological processes of a plant that are positively correlated to RGR, however, there are very important physiological process like photosynthesis and also parts which are responsible for nutrients uptake though studies suggest that plants can still have a maximum growth rate in absence of nutrients but in the presence of light energy.(Hunt and Cornelissen 1997)

RGR depends on plant size and age, plants with different sizes and ages might have different RGRs. RGR increase with plant size until the plant is aged where RGR will be constant and then followed by a drastic decrease in RGR, this implies young plants have higher RGR than adult plants. The factors that decrease plant size also lead to a decrease in RGR, however, increasing in size can also lead to a decrease in RGR due to morphological and physiological functions of a plant (Rees et al.2010; Li et al. 2016).

The reasons to why young plants have high RGR than adult plants is that during the early stages of growth and development, plants develop leaves which play a vital role of capturing sunlight energy for photosynthesis hence resulting into exponential growth. This also implies that plants use their resources differently depending on the stage of growth and environmental conditions. (Tessmer et al. 2013).For example, Hunt and Cornelissen (1997) suggested that plants during their seedling stage show a rapid growth rate after germination which is later followed by a constant growth (i.e. no change in size)and finally growth decline drastically as the plant is becoming mature.

Osone et al. (2008) revealed that RGR and growth parameters vary with plant size and if we are comparing RGR among different plant species, it is better to have plants of the same size, this can be done only when they are given the same period of growth before harvesting.

Different species grown at the same time and given the same requirements can still have varying RGR due to the genetic makeup of some species, this implies that not only phenotypic traits can determine the RGR of a plant but also genotype traits have a role in determining RGR (Poorter and Remkes 1990; Osone et al.2008). However, RGR variation can increase as the variations between species increase (Genetic, phenotypic and physiological levels), when comparing RGR among different species, RGR can also vary depending on the period between harvests. (Poorter and Garnier 1996)

When analysing the components of RGR and their role in its variation, it is very important to deal with a population of many different species (i.e. more than 5 different species). It is also important to measure RGR parameters at different time intervals and at least two successive harvests in order to have a clear comparison. RGR increases drastically in the early stages of the plant (i.e. following germination, followed by a constant increase in RGR with time and finally RGR will decline as the plant is getting mature). (Hunt and Cornelissen 1996).

Differences in RGR among different species are expected even when comparing species from the same genus and grown in the same environmental conditions when the external environment is kept at a constant, variations in RGR among species is expected to be brought up by genetic composition of a plant, morphology and physiology. It is important

to figure out which specific trait is responsible for the variation of RGR among species. (Shipley 2002).

2.3.2. COMPONENTS OF RGR.

2.3.3. SLA. (Specific Leaf Area).

Specific leaf area is the amount of leaf area per unit leaf weight. It is a morphological component of RGR because it depends on the leaf structure (i.e. leaf thickness and leaf dry weight). (Shipley 2002).

SLA is an important and functional trait of a plant that can be used when comparing the variation in the growth rate between species. SLA undergoes plasticity during changes in the environment so that plant continues to function even when the conditions are not at the optimum (i.e. when there is low light intensity, SLA is increased so that the plant can increase the amount of light captured and Carbon dioxide gain for photosynthesis). This implies that plasticity is advantageous not only on organ level but also on a plant trait level. (Lui et al. 2016).

Some parts of the plant like leaves express plasticity in different environments in order to continue functioning even when some resources like water, light and mineral nutrients are not sufficient. SLA also reacts to the changes in nutrient and light availability. (Chen et al.2010). The plant morphologically adjusts its SLA during low intensity of light so that photosynthesis and other important physiological processes can continue (Lui et al.2016).

SLA is one of the components of RGR and it is also an element of Leaf Area Ratio (LAR). They are many factors that can affect the SLA like morphological factors and secondary metabolites. (Lambers and Poorter 1992). However, environmental factors like light availability and humidity can also influence SLA in some plant species. Li et al. (2017) showed evidence that support leaf morphology characteristics to determine RGR where he found out that slow growing plants had tough leaves than fast-growing plants, tough leaves are impermeable to gas (Carbon dioxide) and water entering into the leaf hence leads to a slow rate of photosynthesis.

James and Drenovsky (2007) showed that SLA was directly proportional to RGR in invasive species though was not the only factor for high RGR in invasive species. When comparing RGR of slow and fast growing species based on the rate of photosynthesis, slow

and fast growing species have the same RGR though photosynthetic tissues of fast-growing species are higher due to higher SLA but this dilutes the leaf biomass over the larger SLA which makes the photosynthetic rate per unit leaf area of fast-growing species similar to the one in slow-growing species, therefore SLA and photosynthesis cannot be the only factors to look at when making an inference about the variation of RGR between slow and fast growing species. (Poorter et al.1990).

Osone et al. (2008), suggested that as SLA increases, it may lead to decrease in other RGR components which would otherwise lead to increase in RGR, therefore an increase in SLA does not mean an increase in RGR always.

Apart from specific leaf area, other leaf traits play a very important role in making the entire plant functioning properly because if all leaf traits are modified in a way they adapt to adverse ecological conditions, the leaf as a major important vegetative part of the plant will continue its role in acquiring the resources (i.e. Carbon dioxide and light energy) (Ramírez-Valiente et al. 2017)

The bigger SLA is functionally associated with the amount of resources captured by the leaf in order to perform the basic physiological processes like photosynthesis; this means that SLA increases with the increase in the rate of physiological processes taking place in leaves. (Grotkopp et al.2010)

Despite the fact that many studies suggest that there is a positive correlation between SLA and RGR, there are also many studies that show a poor correlation between SLA and RGR but show a positive correlation with other RGR components. Therefore, the strength of correlation between RGR and its components varies between studies and also depends on some environmental conditions. (Shipley 2006).

2.3.4. Net Assimilation Ratio (NAR).

Net Assimilation Rate is the increase in dry biomass per unit leaf area. (Li et al.2016). NAR is said to be a physiological component because it is a measure the rate of photosynthesis in the whole plant compared to the amount of Carbon captured by leaves hence there is a positive correlation between NAR and resources captured by leaves. (Shipley 2002).

NAR is one of the most vital elements of RGR and is regulated by the amount of Carbon dioxide captured by leaves for photosynthesis and the amount of Carbon lost during respiration. (James and Drenovsky 2007), many publications made about RGR and its components show NAR to be the primary determinant of RGR, however, Lambers and Poorter (1992) suggested that Leaf Area Ratio (LAR) could also be responsible to the variations in RGR because leaves are fundamental vegetative parts of the plants where they act as a site for photosynthesis which implies that if leaves have a large surface area will lead to increase in the rate at which glucose is made hence higher RGR. This can also be supported by Shipley (2006), who concluded SLA to be the primary determinant of RGR which can be reasonable because SLA is also a component of LAR which is both leaf traits. According to the previous publications, NAR would be the primary determinant of RGR as it has a relationship with photosynthesis and respiration which are crucial processes in plant growth. This doesn't mean that other RGR components are not significant but NAR is most correlated to RGR in most cases according to the previous publications. (Li et al.2016).

Though different studies show different RGR components as the main determinants of RGR, NAR would be the primary determinant of RGR regardless of species and environmental conditions because NAR is related to the rate of photosynthesis. (Shipley 2006)

2.3.5. Leaf Area Ratio (LAR).

This is the amount of leaf area per unit total plant weight. LAR is a product of Specific Leaf Area (SLA) and Leaf Weight Ratio.

Thus LAR=SLA*LWR.

The leaf area ratio (LAR) represents useful leaf area for photosynthesis and is the ratio between the area responsible for trapping light energy and CO2 and the total dry matter, therefore LAR is a very important RGR component hence contributes much in the rate at which plants make their own food which will be eventually converted into growth. (dos Santos et al. 2016)

Leaves play a major role in plant productivity hence can influence 90% of the entire plant functioning because it is responsible in capturing of Carbon and convert it to energy used by the entire plant. (Li et al. 2017)

There is a strong correlation between SLA and RGR, from the equation above, it implies that LAR is determined by SLA hence LAR is a prime component that determines RGR, therefore investment in biomass other than leaf area decreases RGR. (Lambers and Poorter, 1992).

To quantify the importance of NAR, SLA and LMR in determining RGR, it was discovered that SLA in herbaceous species was higher than that in woody species though they were no reasons for the variations in SLA between herbaceous species and woody species. This study concluded that though SLA can determine RGR in some species and in some environmental conditions, NAR was the main component that determined RGR, however, the study showed no correlation between LMR and RGR. (Shipley 2006).

Some mineral elements needed by the plants can also determine RGR. Low potassium conditions lowered the LAR which also led to the decrease in RGR during the growth of lettuce. This also implies a positive correlation between LAR and RGR. (Zhang et al. 2017)

2.3.6. Leaf Mass Ratio (LMR).

LMR is defined as the proportion of biomass allocated to leaves. This RGR component was not shown as a major component in determining the RGR. LMR is directly proportional to the leaf thickness which may lower the amount of light captured by the leaf hence lowering photosynthesis which is a major process as far as RGR is concerned. (James and Drenovsky, 2007).

According to Shipley (2006), LMR was not positively correlated with RGR, species don't show a high RGR because of its high LMR, however, it does not mean there is a no relationship between RGR instead the relationship depends on the study, species and environmental conditions.

Leaf mass ratio can be negatively influenced by defoliation which will not only reduce RGR but also affect other physiological plant processes; however, plants that have experienced partial or complete defoliation can have a relatively high RGR though there is

no mechanism explaining how these plants can have a high RGR without leaves. (Anten and Ackerly 2001).

2.4. MEASURING RGR

Relative growth rate is a paramount analytical component of growth analysis and can be measured by two destructive harvests of vegetative parts of individual plants so that initial total dry weight is measured as well as the final dry weight hence RGR and its components are calculated. (Hunt et al.2002; Hoffmann and Poorter, 2002).

When calculating RGR, it is important to measure the entire plant area not just measuring the leaf area, plant area can be measured manually or using computer programs especially when a big population of plants is studied. (Tessmer et al. 2013).

When measuring and comparing RGR among different species (i.e. that differ in nature, genome size, life strategies and geographic location), it is important to use a sufficient number of species (i.e. native, invasive, weedy species, non weedy species, perennial and annual to mention but a few). (Hunt and Cornelissen 1997)

Measuring RGR can also be done by weighing the dry weight of the main parts of the plant using a scale (i.e. leaves, stem and roots) whereby the results determine the RGR of a plant. Total dry weight can also be influenced by environmental conditions (i.e. Carbon dioxide concentration, temperature and Light intensity). (Koca and Erekul 2006)

Taking the biomass measurements of the vegetative parts for comparison is done twice (i.e. first and second harvest). To avoid errors, plant species should be grown in an environment with optimal conditions and individuals should be spaced to avoid competition during growth. (i.e. the conditions for the growing atmosphere must be uniform).(Hunt and Cornelissen 1997)

Most of the measurements taken to infer about the Relative Growth Rate of any plant are taken from the leaf as the major vegetative part of the plant that determines the RGR. RGR can be measured by compiling the measurements of the main components of RGR (i.e. Net Assimilation Rate (NAR), Specific Leaf Area (SLA) and Leaf Mass Ratio (LMR) (Shipley 2006). Measuring RGR is done by using primary data in form of mass, area and volume of the most important vegetative parts (i.e. leaves, stems and roots) of the plant that plays a

role in growth. The input variables should be weight, area and volumes of vegetative parts of the plant plus the date of the first and second harvests (Hunt et al.2002).

Many studies evaluating RGR has compared species from habitats which differ in nutrient contents (i.e. comparing species grown from fertile soil with species in a poor soil) because soil fertility can also determine some of the components of RGR like LAR and SLA hence influencing the entire RGR. Again plant species can have low or high RGR depending on growing conditions, generally plant species tend to have a high RGR when are grown in resource-rich habitat and low RGR when grown in resource-poor environments, however, some species will have variations in their RGR despite grown together in a resource-rich environment (Hunt and cornelissen 1997).

2.5. Factors affecting RGR

2.5.1. Phenotypic plasticity.

Phenotypic plasticity is when plants or other organisms are capable of producing more than one phenotype when exposed to different environmental conditions. (Gratani 2014; Lui et al.2016; Rutherfold et al. 2017).

In general, phenotypic plasticity is very important to the plant because they are sessile, it increases their tolerance during changes in their habitats by developing structures and traits that help plants surviving in adverse environmental conditions hence leading to variation in RGR.(Lui et al. 2016; Rutherfold et al. 2017; Vilas and Pannell 2017).

Although plasticity is important, it can increase invasion because it helps invasive species to cope up with new ecological conditions where they are able to compete, survive and reproduce. There is a positive correlation between polyploidization and phenotypic plasticity because polyploids tend to change their phenotypes when they are in different environmental conditions. The level of phenotypic plasticity in polyploid species is more than that in their diploid counterparts, however, they are no studies compared the degree of plasticity in relation to ploidy levels. (Hahn et al. 2012; Ramirez-Valienre et al.2017).

Ploidy levels are also linked with phenotypic plasticity and resistance to harsh environmental conditions because plants with more ploidy levels (i.e. tetraploids and hexaploids) are more tolerant to some stressful environmental conditions like drought. (Van Laere et al.2010).

Many researchers focus on comparing the degree of plasticity between diploid species and polyploid species and many authors conclude a high degree of plasticity in polyploid species, however, the degree of plasticity among polyploid species vary (i.e. it increases with increase in ploidy levels). (Villas and Pannell, 2017).

Phenotypic plasticity is always meant to improve the performance of plant during unusual environmental conditions but greater SLA during shade was not correlated with improving plant performance instead led to decrease in plant biomass. This was because shade-intolerant plant species produced thin leaves with higher SLA while shade-tolerant species did not possess a significant increase in SLA during shading which later significantly decreased the plant biomass in shade intolerant species. This, therefore, implies that plasticity helps the plant to adapt during shading period but in this case, it led to the decrease in RGR. (Li et al. 2016).

2.5.2. Shoot and root competition.

The relation between the below ground and above ground parts of the plant is important for a plant to carry out all the physiological functions. These competitions determine some physiological processes like reproduction, growth, photosynthesis and development (i.e. root competition has an effect on plant growth and development while shoot competition has an effect on reproduction and photosynthesis because all the parts responsible for those processes are among the shoot system). (Wang et al. 2014)

2.5.3. Ploidy level.

Increase in ploidy levels lead to change in physiology and morphology of a plant (i.e. leaf area, leaf thickness, stomata density), this is because after meiotic cell division there are some genetic changes that may cause phenotypic differences, however, the doubling of the genome size does not result into new genes and chromosomes instead copies of already existing genes and chromosomes. (Van Laere et al. 2010). When comparing the growth rates among species with different ploidy levels, habitat conditions should not be ignored because they also play a role in determining the performance of plant species regardless of their ploidy levels. In some habitats, diploid species show high growth rate than hexaploid species though polyploidy species tend to have high performances than diploid species in general. (Černa and Münzbergová, 2013).

2.5.4. Carbon dioxide concentration and the rate of photosynthesis.

Plants grown in conditions with elevated Carbon dioxide concentration tend to have a higher RGR than those grown in conditions with low Carbon dioxide concentration because Carbon dioxide is an element of photosynthesis that play a role in plant functioning and contributes much to the increase in the shoot system, however, it may lead to poor absorption of Nitrogen by roots hence plants lacking Nitrogen (i.e. nutrients, amino acids and proteins) may wither and eventually die. Plants grown on places with high elevated Carbon dioxide tend to have a high leaf area but lower mass, this leads to increase in the rate of Photosynthesis hence higher RGR. (Ishizaki et al. 2003)

Photosynthesis is a paramount physiological process in plants and plays a very important role in determining plant growth rate, photosynthesis takes place in all the vegetative parts of the plants but most especially in the leaves and they are regarded as the site for photosynthesis.

Native species have a higher RGR than their native counterparts due to their higher SLA which determines LAR because SLA is a component of LAR.(James and Drenovsky, 2007), however, Li et al. (2016) claimed that the main predictor for RGR is NAR as the measurement of photosynthesis in a leaf which indicates that photosynthesis is directly proportional to RGR. Plant traits that are related to area determine the rate of photosynthesis which is a major process as far as RGR is concerned while mass related traits have nothing to do with photosynthesis (i.e. LMR has no role in the variation of RGR). (Li et al. 2016).

Another factor affecting the rate of photosynthesis is leaf thickness whereby very thick leaves hinder diffusion of Carbon dioxide hence there will be no Photosynthesis or it will be on a very low rate, on the other hand, very thin leaves also lose water which is also a very important compound for Photosynthesis. In this case, a leaf with a medium thickness is suitable for Photosynthesis hence high RGR. (Ishizaki et al. 2003)

Poorter et al.(1990), suggested that Photosynthesis should not be the only factor to be considered when analysing crop yield and RGR because photosynthesis is just one of the physiological process of the plant which plays a role in plant growth and functioning.

2.5.5. Mode of reproduction.

Vegetative reproduction is a quick type of reproduction as it does not involve complicated processes like pollination and fertilization; polyploidy species have a high RGR due to the fact that many polyploidy species rely on asexual reproduction as their type of reproduction. Apart from leading to high RGR, asexual reproduction can also lead to increase in plant population in a short time, however, offsprings are identical to each other and to the parents which cause lack of genetic variability. (Herben et al. 2016)

Diploid plants that reproduce sexually have a lower RGR than asexual polyploidy species hence polyploidy species have a short maturation period, however, some studies revealed the slow growth rate in polyploidy species because of the increased genomic size which takes part in slowing the metabolic rate hence slow growth rate at some point. (Larkin et al. 2015).

2.5.6. The effect of nutrients.

Nutrient availability determine RGR as amino acids contribute to the development and growth of major vegetative structures which also influence RGR, however, nutrients should be regulated such that the plant cannot be provided with excess nutrients which will results in plant stress hence limiting the proper functioning of the plant (Scheirs and De Bruyn, 2004). Some types of nutrients supplied to plants may have an influence on some of the major components of RGR (i.e. NAR and LAR) that have a positive correlation with RGR, on the other hand, some nutrients can be toxic even if they are supplied in a larger amount. (Kabay et al. 2017).

In general nutrients supply is directly proportional to RGR because RGR increases when new structures are developed hence species with a good supply of nutrients have a high RGR than those that are grown in poor nutrient supply. (dos Santos et al. 2016) In most cases, nutrient supply increase with RGR among species though some *Chenopodium* species can continue to have a high RGR due to their high capacity to resist during stressful condition or due to other factors other than nutrients supply. (Shipley and Keddy 1988).

Villas and Pannell (2017), suggested that when appropriate nutrients are supplied to plants can increase both the underground biomass (roots) and the above the ground biomass (the shoot system). Plant species whose habitats are fertile tend to have a high RGR than plant

species whose habitats are not fertile (i.e. in most plant species growth cannot exist in a nutrient-poor soil because it will make their RGR decreasing drastically). (Shipley and Keddy 1988).

Potassium is one of the major mineral elements that play a role in plant growth as it is involved in all the important physiological processes of a plant; studies showed that RGR and yield can be determined by the amount of Potassium supplied to a plant. Photosynthesis is one of the physiological processes that are influenced by mineral nutrients like Potassium as it regulates the opening and closing of stomata (i.e. regulates Carbon dioxide uptake, activation of enzymes responsible for starch synthesis and production of ATP for translocation of sugar to all parts of the plant, to mention but a few). (Zhang et al. 2017).

Bhargava et al. (2005), suggested that although *Chenopodium* species like *Chenopodium quinoa* responds well to Nitrogen nutrients, its yield and growth can be lowered by an excessive supply of Nitrogen fertilizers.

CHAPTER THREE

3. METHODOLOGY (Materials and Methods)

3.1. Study Species:

In this research, 8 *Chenopodium* species with different ploidy levels were used and were collected from three different countries in different localities: Czech Republic, Greece, and China. Two hexaploids, two diploids and 4 tetraploids.

Table 1: 8 species from different ploidy levels and countries from which they were collected.

SPECIES	PLOIDY LEVEL	COUNTRY OF ORIGIN
C. album	hexaploid	Czech Republic
C. ficifolium	diploid	Czech Republic
C. striatiforme	tetraploid	Czech Republic
C. strictum	tetraploid	China

C. acuminatum	diploid	China
C. karoi	tetraploid	China
C. novopokrovskyanum	tetraploid	China
C. opulifolium	hexaploid	Greece

3.2. Study area

The experiment of comparing the relative growth rate (RGR) among different *Chenopodium* species with different ploidy levels was carried out at the Czech University of Life Sciences-Prague (CULS), Czech Republic 50.1300°N, 14.3734°E.

The present study was conducted in the Czech Republic on materials collected from three different countries (i.e. Czech Republic, China and Greece).

Table 2: Details and characterization of the localities in each country where seeds were collected.

COUNTRY	LOCALITY	GPS	AVERAGE ANNUAL TEMPERATURE	AVERAGE ANNUAL PRECIPITATION
Czech Republic	Hrádek	48.781583N, 16.261528E	7.3°C	775 mm
	Rejšice	50.319972N, 14.978806E	8.6 °C	524 mm.
	Slatina	50.2263889N, 14.210528E	8.1 °C	589 mm.
	Mělnik	50.349527N, 14.497444E		179mm
China	Xinjiang/Altaj	46.991916N, 89.538889E	3.6 °C	179mm

	Xinjiang/Altaj	46.962722N, 89.627333E	3.6 °C	179mm
	Xinjiang/Altaj	47.926556N, 88.136917E	3.6 °C	179mm
	Xinjiang/Tumuxiukezhen	41.667306N, 79.693528E	3.6 °C	179mm
	Xinjan/Altaj	48.034056N, 86.881667E	3.6°C	179mm
Greece	Crete island	35.245312N, 24.47001E	18.9 °C	523 mm

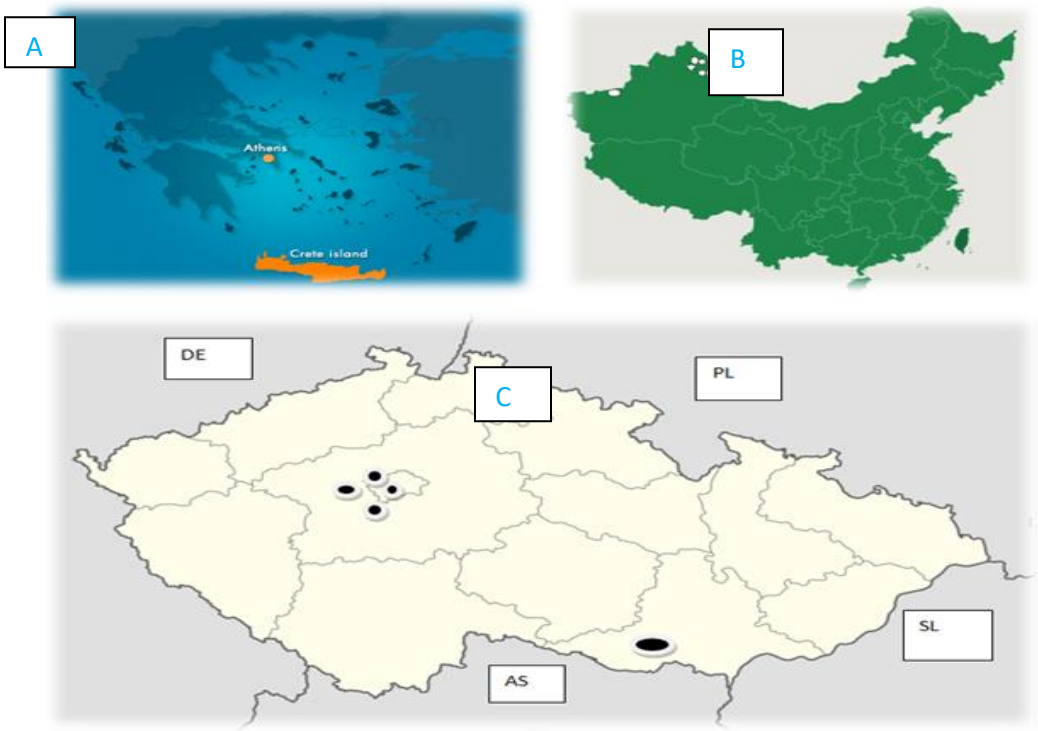

Fig.7. Map of Czech Republic (**C**), Greece (**A**) and China (**B**) showing different localities where seeds were collected.

3.3. Breaking the seed dormancy and germination

Cleaning of the seeds was done manually by scratching and removing the dust and seed coats as one of the methods of breaking the seed dormancy, however, we finally used the cold stratification method for all *Chenopodium* species to completely break the seed dormancy so that they will germinate at the same time. All experiments were conducted at the laboratory of Czech University of Life Sciences, Faculty of Environmental Sciences.

All the seeds were put in Petri dishes (i.e. 2 Petri dishes were used for each sample, a small petri dish were put into the bigger one so as to hold the filter papers where the seeds were spread). Filter papers were moistened with distilled water and finally, the seeds were spread on the filter papers. All Petri-dishes were wrapped in Aluminium foils so as to keep them out of light. Wrapped Petri-dishes were placed in the refrigerator (cold stratification at 5°C) for 14 days.

After 14 days, all Petri dishes were removed from the refrigerator. They were unwrapped and placed into a growing chamber. The regime in the growing chamber was set as the ISP standard temperature was 22°C by day and 15°C by night, the ISP standard temperature, i.e. 22°C and 15°C night, 14 hours light and 10 hours dark. Humidity was set at 70%.

All the plant species were grown in a growing chamber and were given uniform conditions for the comparison of RGR. (Wright and Westoby 2000; Sugiyama 2005).

3.4. Transplanting of the seedlings

Transplanting of germinated seedlings started after 3 days from the date of introducing the seeds into the favourable conditions for germination. Germinated seedlings were transplanted into pots full of perlite substrate, a porous substance which is excellent for water retention and seedlings were bottom and top watered by Hoagland's solution every three days. Perlite substrate was mixed with Hoagland's solution which is rich in both micro and macronutrients, the major components of the solution are: KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$, $MgSO_4 \cdot 7H_2O$, $ZnSO_4 \cdot 7H_2O$, H_3BO_3, $CuSO_4 \cdot 5H_2O$, $Na_2MoO_4 \cdot 2H_2O$. Hoagland's solution was used for both as a nutrient supply solution and for moisturizing the perlite substrate during the transplanting of the seedlings in the pots.

Fig. 8: The growing chamber where plants were grown.

3.5. Plant harvesting and processing

Harvesting was done into 2 phases, the first harvesting was done 21 days after transplanting the seedlings into growing pots and it was done by washing the plants to remove all the substrate and cutting the plants using a pair of scissors to separate the vegetative parts (leaves, stem and roots). The second harvest was done after 14 days of the first harvest.

Fig.9: Plants removed from growing chamber ready to be harvested.

3.6. Scanning the leaves

During harvesting, leaves were scanned using Hp Scanjet 5530 Photosmart scanner so that the leaf area (LA) can be processed by ImageJ 1.4.3 67 software to measure the LA because it is a paramount parameter in studying the plant growth as well as RGR. LA as it is related to very important physiological processes like photosynthesis, transpiration and gaseous exchange, however, it is used to calculate morphological RGR components (i.e.Specific leaf area and Leaf Area Ratio).

3.7. Drying the plant parts

The main reason for drying the plant parts was to measure the plant dry weight. After harvesting, plant parts were kept for 3 weeks for drying and finally oven-dried at 60°C for 24 hours before measuring them.

3.8. Measuring leaf, stem and root weight

This was done using Sartorius 1 analytical scale, the weight for all vegetative parts was measured in grams. Leaf, stem and roots weight were measured and recorded separately for first and second harvest.

3.9. Calculating the RGR components

RGR components were calculated directly by using the mass, leaf area and the harvest time as all individuals were of the same age but varied in size. (Li et al.2016).

Calculations for growth analysis parameters (i.e. RGR, SLA, ULR, LMR and LAR). (Rees et al.2010).

$$\mathbf{RGR} = \frac{\boldsymbol{DW_2 - DW_1}}{(\mathbf{t_2 - t_1})}$$

Where $\boldsymbol{DW_2}$ and $\boldsymbol{DW_1}$ are dry weights for the second harvest and first harvest respectively, $\mathbf{t_2}$ and $\mathbf{t_1}$ are time in days for final and initial harvest respectively.

Specific leaf area is the ratio of leaf area to leaf mass

$$\mathbf{SLA} = \frac{\boldsymbol{LA_1}}{\boldsymbol{LW_1}} + \frac{\boldsymbol{LA_2}}{\boldsymbol{LW_2}}$$

Where $\boldsymbol{LA_1}$and $\boldsymbol{LA_2}$ are leaf areas for initial and final harvests respectively while $\boldsymbol{LW_1}$ and $\boldsymbol{LW_2}$ are leaf masses for initial and final harvests respectively.

Net assimilation rate is mass increase per unit leaf area.

$$\mathbf{NAR} = \frac{\boldsymbol{DW_2 - DW_1}}{\boldsymbol{LA_2(t_2 - t_1)}}$$

Leaf mass ratio is the ratio of leaf mass to total plant mass.

$$\mathbf{LMR} = \frac{\boldsymbol{LW_1}}{\boldsymbol{DW_1}} + \frac{\boldsymbol{LW_2}}{\boldsymbol{DW_2}}$$

Leaf area ratio is the ratio of leaf area to plant mass.

$$\mathbf{LAR} = \frac{\boldsymbol{LA_1}}{\boldsymbol{DW_1}} + \frac{\boldsymbol{LA_2}}{\boldsymbol{DW_2}}$$

3.10. Statistical analysis

A one-way analysis of variance (ANOVA) was used to evaluate the differences in mean of *Chenopodium* species, different ploidy levels, *Chenopodium* species from different clades, and comparison of RGR among invasive and noninvasive *Chenopodium*. In the case of significant result Tukey (HSD) *posthoc* test was used.

All statistical analysis was performed using STATISTICA 12.0 program (www.statsoft.com).

CHAPTER FOUR

4. RESULTS

In all 40 individuals from 8 *Chenopodium* species (Tab.3) were collected and analysed for their Relative Growth Rate (RGR). Except for RGR and NAR in connection with *Chenopodium* species. Meanwhile, Leaf Mass Ratio (LMR) (Fig.15.), Specific Leaf Area (SLA) (Fig.14) and Leaf area Ratio (LAR) (Fig.16) expressed significant differences among *Chenopodium* species.

The highest average RGR/day was recorded in *C.novopokrovskyanum* (0.0098), followed by *C. stratiforme, C.album, C.strictum, C.opulifollium, C.acuminatum, and C.ficifolium*. The lowest RGR/ day was valued at 0.003 with *C karoi.* In descending order the Specific Leaf Area (SLA) ranged between 72.734 cm^2/g in *C strictum* and 6.05 cm^2/g in *C karoi* (Fig.10). The highest Leaf Area Ratio (LAR/cm^2/g) was identified with *C.novopokrovskyanum* (28.737) which is followed by *C.strictum* (27.548), *C.album* (15.411), *C.ficifolium* (12.811), *C.opulifollium* (9.909), *C.stratiforme* (7.881), and *C.acuminatum* (3.785). The lowest (LAR/cm^2/g) was recorded with *C.karoi* valued at 1.962 (Fig.16). The highest ploidy level was recording 4x followed by 6x and 2x recording the least value.

Table.3. Total number of individual species taken from different populations in the the Czech Republic, China and Greece with a brief description of the individual population.

COUNTRY	POPULATION	POPULATION DESCRIPTION	SPECIES	No of individuals
Czech Republic	Hrádek	Exposed ruderal place	*C.album*	4
Czech Republic	Rejŝice	Exposed pond bottom	*C.ficifolium*	4
Czech Republic	Slatina	Dungheap in the field	*C.album*	3
Czech Republic	Slatina	Dungheap in the field	*C.ficifolium*	3
Czech Republic	Mělnik	Base of the wall railway station	*C.stratiforme*	9
China	Xinjiang, Altaj	Ruderal places along the road	*C.strictum*	3
China	Xinjiang, Altaj	Eroded and grazed margins.	*C.acuminatum*	2
China	Xinjiang, Altaj	Along a cattle-pen	*C.karoi*	3
China	Xinjiang, Tumuxiukezhen	Dry places along the road	*C.novopokrovskyanum*	3
China	Xinjiang, Altaj,Burqin	Sands and sand dunes along the road	*C.acuminatum*	2
Greece	Crete island	Wasteland	*C.opulifulium*	4

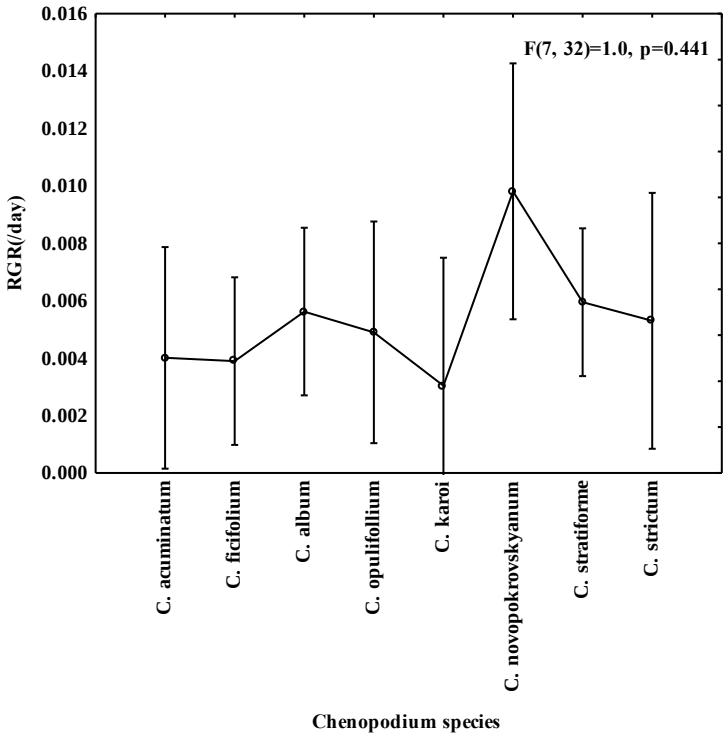

Fig. 10: Effect of *Chenopodium* species on Relative Growth Rate (RGR/day). F and P values were obtained by One-way analysis of variance (ANOVA).

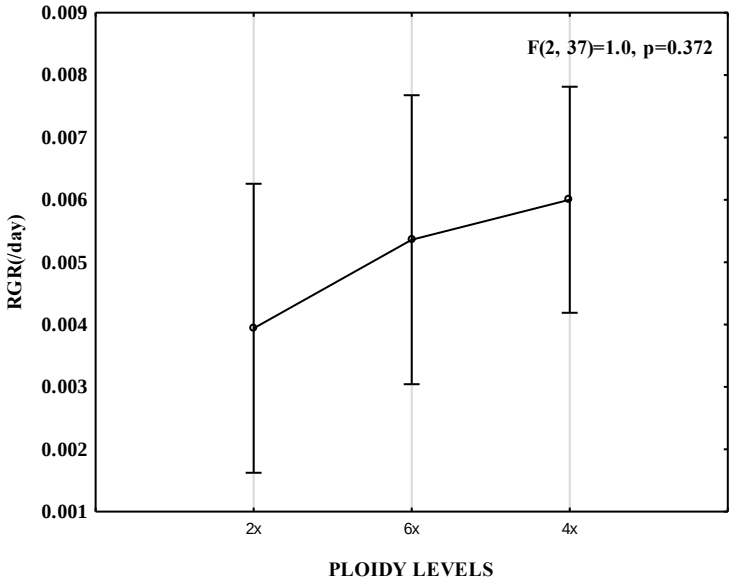

Fig. 11: Effect of ploidy levels on Relative Growth Rate (RGR/day). F and P values were obtained by One-way analysis of variance (ANOVA).

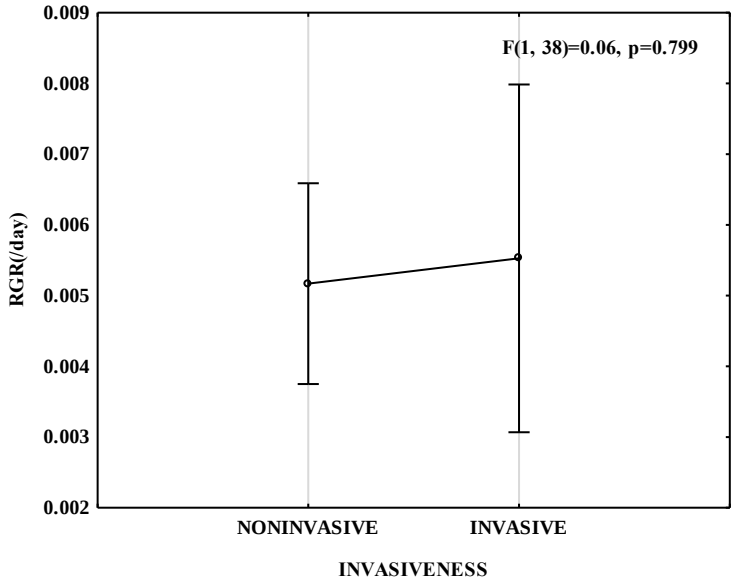

Fig. 12: Effect of Invasiveness (Noninvasive and Invasive) on Relative Growth Rate (RGR/day). F and P values were obtained by One-way analysis of variance (ANOVA).

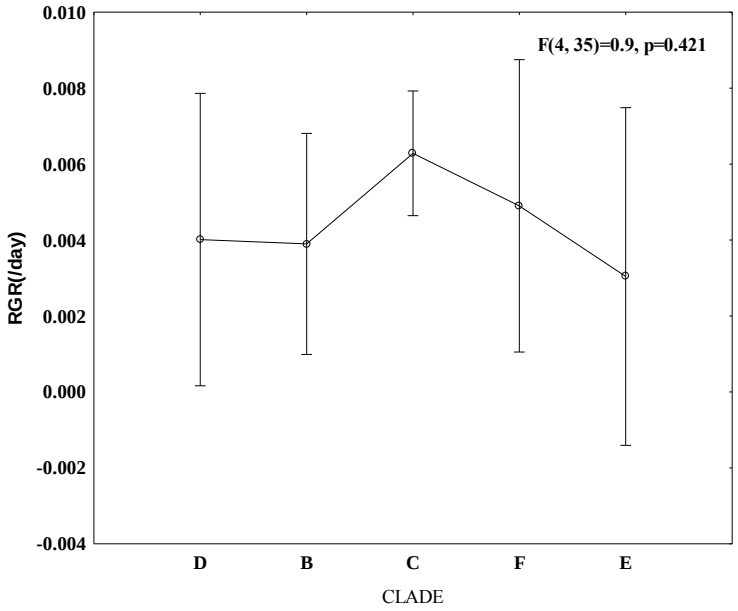

Fig. 13: Effect of phylogeny on Relative Growth Rate (RGR/day). F and P values were obtained by One-way analysis of variance (ANOVA).

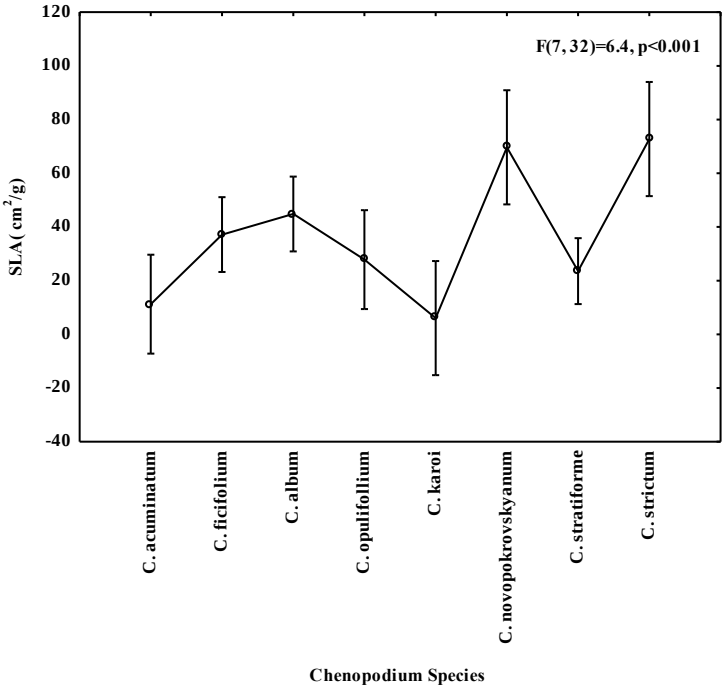

Fig.14: Effect of *Chenopodium* species on Specific Leaf Area (SLA/cm^2/g). F and P values were obtained by One-way analysis of variance (ANOVA).

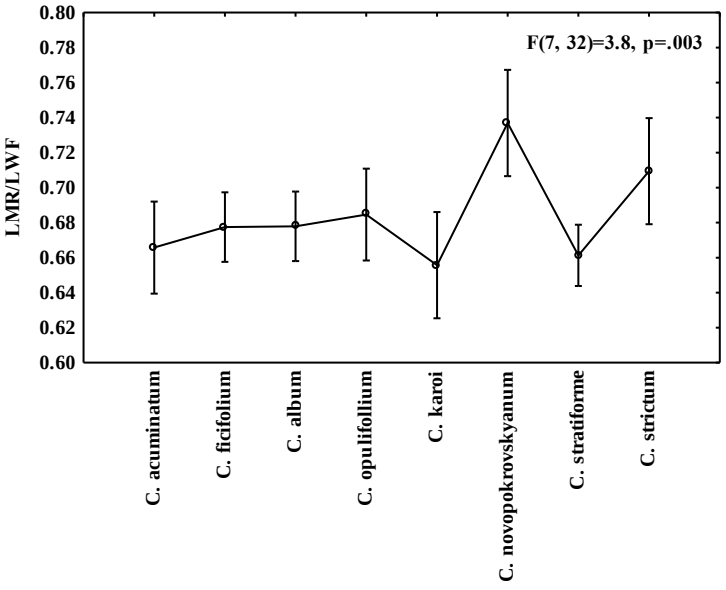

Fig .15: Effect of *Chenopodium* species on Leaf Mass Ratio (LMR/LWF). F and p values were obtained by One-way analysis of variance (ANOVA).

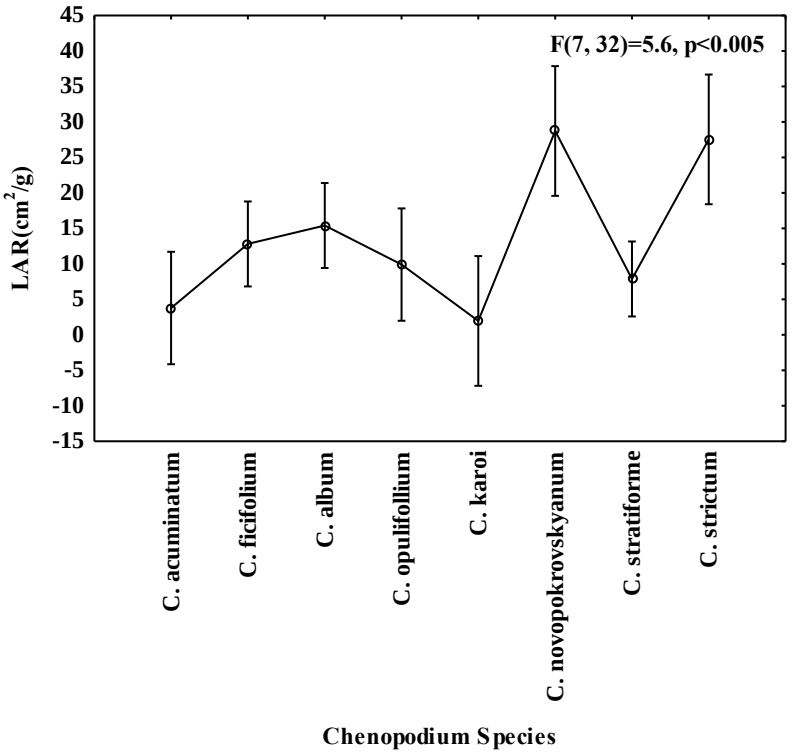

Fig .16:.Effect of *Chenopodium* species on Leaf Area Ratio (LAR/cm^2/g). F and p F and p values were obtained by One-way analysis of variance (ANOVA).

Table.4. Tukey (HSD) *posthoc* test for the comparison of SLA among *Chenopodium* species with significant difference of mean SLA in all *Chenopodium* species. A stronger significance different was identified among *C. filifolium* and *C. album* .

Cell No.	Tukey HSD test; variable SLA(cm2/g) (main data) Homogenous Groups, alpha = .05000 Error: Between MS = 326.75, df = 32.000 SPECIES NAME	SLA(cm2/g) Mean	1	2	3
5	*C. karo*	6.0559	****		
1	*C. acuminatum*	11.2286	****		
7	*C. stratiforme*	23.5535	****		
4	*C. opulifollium*	27.8456	****	****	
2	*C. ficifolium*	37.1517	****	****	****
3	*C. album*	44.8382	****	****	****
6	*C. novopokrovskyanun*	69.6329		****	****
8	*C. strictum*	72.7342			****

Table.5. Tukey (HSD) *posthoc* test for the comparison of LMR among *Chenopodium* species with significant difference of mean LMR in all *Chenopodium* species. A stronger significance different was identified among *C. opulifollium* and *C.strictum.*

Cell No.	Tukey HSD test; variable LMR/LWF(no units (main data) Homogenous Groups, alpha = .05000 Error: Between MS = .00067, df = 32.000 SPECIES NAME	LMR/LWF(no units Mean	1	2
5	*C. karo*	0.65570	****	
7	*C. stratiforme*	0.66129	****	
1	*C. acuminatum*	0.66575	****	
2	*C. ficifolium*	0.67746	****	
3	*C. album*	0.67791	****	
4	*C. opulifollium*	0.68459	****	****
8	*C. strictum*	0.70939	****	****
6	*C. novopokrovskyanun*	0.73692		****

Table. 6. Tukey (HSD) *posthoc* test for the comparison of LAR among *Chenopodium* species with significant difference of mean LAR in all chenopodium species. A stronger significance different was identified among *C.opulifollium, C.ficifolium and C.album.*

Cell No.	SPECIES NAME	LAR(CM2/g) Mean	1	2
	Tukey HSD test; variable LAR(CM2/g) (main data) Homogenous Groups, alpha = .05000 Error: Between MS = 60.443, df = 32.000			
5	C. karoi	1.96283	****	
1	C. acuminatum	3.78530	****	
7	C. stratiforme	7.88131	****	
4	C. opulifollium	9.90980	****	****
2	C. ficifolium	12.81167	****	****
3	C. album	15.41198	****	****
8	C. strictum	27.54843		****
6	C. novopokrovskyanum	28.73723		****

CHAPTER FIVE

5. DISCUSSION

The present study shows only marginal variation in RGR among *Chenopodium* species. Statistical analysis showed no significant differences in RGR among *Chenopodium* species, however, *Chenopodium* species with higher ploidy levels but not all expressed quiet higher RGR than diploid counterparts.

Relative growth rate is decomposed of various components that are both morphologically and physiologically based. RGR is the product of specific leaf area (SLA), net assimilation rate (NAR) and leaf mass ratio (LMR) (Lambers and Poorter 1992; Hunt et al.2000; Wright and Westoby 2000; Shipley 2000; Osone et al. 2008; Li et al.2016). Calculating RGR involves destructive harvests in order to measure plant dry weight, biomass, leaf area, leaf weight, stem weight, root weight, basal area and stem diameter. (Pommerening and Muszta 2015).

Results of this study are opposing other comparative studies in relation to ploidy levels that have always indicated that plant species with higher ploidy levels have traits that activate their function which is mainly associated with the increased genome size, however, if habitat conditions are taken into account, they can also have an influence on the species functions regardless of its genomic size (Černa and Münzbergová 2013).

Many studies that assessed the variation of RGR among species have only been comparing the effect of different ecological conditions, native and invasive species, species grown in poor soils against species grown in nutrient-rich soils, there are few studies that have compared the RGR among species with different ploidy levels, others are just comparative studies that asses which RGR component strongly correlates with RGR. (James and Drenovsky 2007).

Other studies, for example, Oguchi et al.(2015) who analyzed the variation in RGR among plant species, compared species from different races and from different habitats and the results showed significant differences in RGR among species.

Statistically the results of the present study showed no difference in RGR among *Chenopodium* species, however, some RGR components showed significant differences among *Chenopodium* species.

5.1. The contribution of RGR components

In order to acquire a maximum RGR, both below ground and above ground parts have to contribute, however, morphological RGR components with parts mainly above ground parts always expressed a strong correlation with RGR. (Hunt and Cornelissen 1997).

Morphological and physiological factors in plants influence the variation of RGR among different species and it is important to know that not only genetic factors but also environmental factors influence in the variation of RGR among species for instance if RGR is compared among species grown in a habitat with maximum light availability and a habitat with low light availability, plant species grown in an environment with maximum light will have a higher RGR regardless of the genome size.

In the present study, three RGR components SLA, LAR and LMR expressed significant variations among species and they were quite related to RGR, however, we did not perform any correlation analysis for RGR components with RGR. For instance, species which expressed a high mean SLA also expressed a higher RGR compared to other species (i.e. *C.novopokrovskyanum* expressed a higher SLA (69.632) and a quiet higher RGR (0.0098 day^{-1} when compared to other species), the species with the lowest SLA *C.karoi (*6.055 cm^2/g) also had the lowest RGR (0.0030 day^{-1}). Another RGR component that expressed a significant variation among species was LAR and was also marginally related to RGR where *C.novopokrovskyanum* had the highest LAR hence expressed the highest RGR when compared with other species.

dos Santos et al.(2016) revealed that NAR as a physiological RGR component that plays an important role in the variation of RGR among species, however, the findings of the present study showed no significant differences in NAR and RGR among *Chenopodium* species. This shows that NAR was directly proportional to RGR(i.e. as the mean NAR is low among species, RGR will also be low.

Therefore RGR components are among the factors that led to almost similar mean RGR among *Chenopodium* species, however, the studies where NAR determined RGR, for example, Li et al.(2016), their experimental conditions where far different from the one in the present study.

In general, the findings of the present study are not in line with most of the other results from previous works that compared growth rates in relation to ploidy levels because their findings showed that species with higher ploidy levels expressed a higher RGR than diploids, however, higher RGR in polyploids depend on other environmental factors. (Černa and Münzbergová 2013).

Oguchi et al.(2015) showed variation of RGR among species, however, in this study there are factors, for example, Carbon dioxide concentration and the amount of Nitrates were considered and they also influenced RGR while in the present study there were no factors considered as all species where grown in uniform environmental conditions and supplies with the same amount of nitrates.

Despite the fact that there was no significant difference in RGR among *Chenopodium* species in the present study, having variation in RGR among species is a common phenomenon in plants. The reason for the results of the present study may be because of the small number of species which also led to small number of individuals and this was caused by the failure to germinate of some *Chenopodium* species. Another reason for the results might be because of the uneven number of individuals from different species and different ploidy levels.

CHAPTER SIX

6. CONCLUSION

The main results of the present study showed no significant differences among RGR among *Chenopodium* species in relation to ploidy levels. This is contrary to our main hypothesis that species with higher ploidy levels will express a higher RGR.

Despite the fact that RGR did not vary significantly among species and ploidy levels in general, three polyploids *C.novopokrovskyanum, C.album* and *C.stratiforme* expressed a quiet high RGR compared to other *Chenopodium* species.

The findings of the study also revealed that the effect of invasiveness on RGR was not significant (i.e. there was statistically no significant difference in RGR among invasive and noninvasive species) and this was also contrary to our second hypothesis that invasive species will express a higher RGR.

RGR components but not all expressed a significant variation among species, however, we did not do any correlation analysis of RGR components with RGR. Morphological and leaf trait components SLA and LAR showed a significant variation among species and they were somehow related to mean RGR (i.e. *C.karoi* expressed the lowest SLA and LAR hence had the lowest RGR compared to other species)

The present study together with previous studies that compared the influence of RGR components on RGR show that all RGR components are important but cannot have an influence on RGR at the same time, their influence depends on the type of species and ecological conditions.

Despite the fact that the findings in the present study did not show a significant difference in RGR among species and polyploids did not express a very higher RGR compared with diploids, increase in genome size is both ecologically and evolutionary important because polyploid species can adapt very well in adverse environmental conditions than their diploid counterparts. (te Beest et al.2012)

REFERENCES

Adam K.L, Wendel J.F. 2005. Polyploid and genome evolution in plants. Current opinion in plant Biology. 8:135-141.

Alix K, Gérard PR, Schwarzacher T, (Pat) Heslop-Harrison JS.2017. Polyploid and interspecific hybridization: Parterners for adaptation, speciation and evolution in plants. Annals of Botany 120:183-194, 2017. Doi:1093/aob/mcx/079.

Anten NPR, Ackerly DD. 2001. A new method of growth analysis for plants that experience periodic losses of leaf mass. Functional ecology 15, 804-811.

Bhargava A, Shukla S, Kumar R, Ohri D. 2009. Metroglyph Analysis of morphological variation in Chenopodium SPP. World Journal of Agricultural sciences. 5(1):117-120.

Bhargava A, Shukla S, Ohri D, 2005. Karyoptic studies in some cultivated and wild species of Chenopodium. Genetic resources and crop evolution (2006) 53:1309-1320.doi:10.1007/510722-005-3879-8.

Bhargava A, Shukla S, Ohri D. 2005. Chenopodium quinoa. An Indian perspective. Industrial crops and products 23(2006) 73-87.doi:10.1016/j.indcrop.2005.04.002.

Bhargava A, Shukla S, Ohri D.2010. Short communication mineral composition in foliage of some cultivated and wild species of *Chenopodium.* Spanish J. of Agricultural research 2010 8(2), 371-376. ISSN: 1695-971-X.

Davidson AM, Jennions M, Nicotran AB. 2011. Do invasive species show higher Phenotypic plasticity than native species and if so, is it adaptive? A meta-analysis. Ecology letters, (2011) 14:419-431.doi: 10.1111/j.1461-0248.2011.01596.x

Cavender-Bares J. 2017. Population –Level Differentiation in Growth Rates and Leaf traits in seedlings of the Neotropical Live Oak Quercus Oleoides Grown under Manipulated Precipitations Regimes. Frontiers in plant science. 8:585.doi:10.3389/fpls.2017.00585. Seville, Spain

Černa L, Münzebergová Z. 2013. Comperative population Dynamics of diploid and hexaploid populations of a herb. Annals of Botany 100:1259-1270, 2007. Doi:1093/aob/mcm 204.oxford journals.

Chen F.S, Zeng D-H, Fahey TJ, Yao CY, Yu ZY. 2010. Response of leaf anatomy of *Chenopodium acuminatum* to soil resource availability in a semi-arid grassland. Plant ecology 209:375-382. Doi 10.1007/s11258-010-9778-X.

Cole MJ. 1961. Interspecific relationships and intraspecific variation of *Chenopodium album* L. in Britain. (The taxonomic delimitation of the species).

Comai L.2005.The advantages and disadvantages of being polyploidy. Nature publishing group. Doi:10:1038/nrg1711.volume 6.

Córcoles JI, Domiguez A, Moreno MA, Ortega JF, deJuan JA.2015. A non-destructive method for eliminating onion leaf area. Irish journal of agriculture and food research.doi:10.15151.ijafr-2015-0002.IJAFR.54(1).2015.17-30

Fuentes-Bazan S, Mansion, G, Borsch, T. 2012. Towards a species level tree of the globally diverse genus (Chenopodium (Chenopodiaceae). Mol Phylogenet Evol. 2012 Jan; 62(1): 359-74.doi:1016/j.ympev.

Gangopadhyay G, Das S, Mukherjee KK. 2002. Speciation in *Chenopodium* in west Bengal India. Genetic resources and crop evolution 49:503-510, 2002.

Geng Y, Dan X, Xu C, Zhang W, Li B, Chen J. 2006. Plasticity and ontogenetic drift of biomass allocation in response to above and below ground resource availabilities in perennial herbs: A case study of Alternanthera philoxeroides. The ecological society of Japan. Doi 10.1007/s11284-006-9. EcoiReso(2007) 22:225-260.

Gohar AA, Maatoq GT, Niwa M, Yoshiaki T. 2002. A new triterpen saponin from *Chenopodium ficifolium*. Naturforsch. 57c.597-602(2002).

Gratani L.2014. Plant phenotypic plasticity in response to environmental factors. Advances in Botany. http://dx.doi.org/10.1155/2014./208747.

Grotkopp E, Erskine-OGDEN J.Rejmanek M, 2010. Assesing potential invasiveness of wood hoilticultural plant species using seeding growth rate traits. Journal of applied ecology. 2010. 47,1320-1328.. doi:1111/J 1365-2264.2010. 01878.X

Grotkopp E, Rejmánek M. 2007. High seedling Relative growth rate and specific area are traits of invasive species phynogenetically independent contrasts of woody angiosperms. American journal of botany 94(4): 526-532.

Hahn MA, Van Kleunen M, Müller-Schärer H.2012. Increased Phenotypic plasticity to climate may have boosted the invasion success of polyploid Centaurea stoebe.PLOS ONE 7(11):e50284. doi:10.1371/journal.pone.0050284

Herben T, Suda J, Klimesova J. 2016. Polyploid species rely on vegetative reproduction more than diploids! A re-examination of the old hypothesis. Annals of Botany 120:341-349, doi:10.1093/aob/mcx 009.

Hoffman WA, Poorter H. 2002. Avoiding Bias in calculations of relative growth rate. Annals of Botany. 80:37-42, doi:1001093/aob/MCF140.

Huang Y, Zhao X, Zhou D, Wang T, Li G, Li Q. 2013. Biomass allocation to vegetative and reproductive organs of *Chenopodium acuminatum* wild. Under soil nutrient and water stress. Bangaladesh journal of botany 42(1):113-121,2013(June).

Hunt R, Causton DR, Shipley B, Askew AP. 2002. A modern tool for classical plant growth analysis. Annals of botany. 90:485-488. Canada J1k ZR1.

Hunt R, Cornelissen JHC. 1997. Components of relative growth rate and the interactions in 51 temperate plant species. New phytologist. Vol 395-417.

Ishizak S, Hikobaka K, Hirose T. 2003. Increase in Leaf mass per area benefits planted growth at elevated Carbon dioxide concentration. Annals of Botany 91:905-914. doi:10.1093/aob/mc9 097. Japan.

James J J, Drenovsky RE. 2007. A basin for Relative Growth Rate Differences between Native and Invasive Fors seedlings. Rangeland Ecol manage 60: 395-400

Jiri R, Leiter GC, Tomas K.2015. The influence of pathogenic organism on growth and production of *Chenopodim quinoa* wild. Under conditions of Czech republic . Mendel Net.

Kabay T, Erdinc C, Sensory S. 2017.Effects of drought stress on plant growth parameters, membrane damage index and nutrient content in common bean

genotypes. The journal of animal and plant sciences.27(3). 940-952. ISSN: 1018-7081.Turkey.

Kakanova-Nedialkova Z, Nedialkova PT, Nikolov SD. 2009. The genus *Chenopodium:* Phtochemistry, Ethnopharmacology and pharmacology. Pharmacognosy reviews. Vol, 3, issue 6. 280-306, 2009.

Karushinge NS, Agarwal AA. 2005. Phenotypic Plasticity to light competition and Herbivory in Chenopodium Album(CHENOPIACEAE). American Journal of Botany. 92(1):21-26.

Koca YO, Erekul O. 2016. Changes in dry matter, Biomass and Relative growth rate with different phonological stages of corn. Agriculture and Agricultural Science Procedia 10(2016) 67-75. Turkey.

Kolano B.Siwińska D. Maluszyńska J.2007. Comparative cytogenetic analysis of diploid and hexaploid *Chenopodium album.* Agg. Acta societatis Botanicorum poloniae. Vol.77,No.4:293-298.

Krak K, Vít P. Belyayev A, Douda J, Hreusová L, Mandák B. 2016. Allopolyploid origin of *Chenopodium S.*Str. (Chenopodiaceae): A molecular and cytogenetic insight. Plos ONE 11(8): e0161063. Doi:10.1371/Journal. Pone. 0161063.

Laere KV, França SC, Vansteenkiste H, Huylenbroeck JV, Steppe K, Labeke MCV. 2011. Influence of ploidy level on morphology, growth and drought susceptibility in Spathiphyllum wallisii. Acta Physiol plant(2011) 33:1149-1156.

Lambers H, Poorter H. (1992). Inherent variation in Growth Rate between Higher Plants. A search for Physiological causes and Ecological consequences. Advanced in Ecological research. Volume 23, pages 187-261.

Larkin K, Tucci C, Neiman M. 2015. Effects of polyploidy and reproduction mode on life history trait expression. Ecology and evolution. 6(3) 765-778. Doi:10.1002/ece3.1934.

Li X, Schmid B, Wang F, Paine CET. 2006. Net assimilation rate Determines the growth rate of 14 species of subtropical forest trees. PLOS ONE 11(3): E0150644. Doi:10.1371/Journal.pone 0150644.

Li Y, Kröber W, Bruelheide H, Härdtle W, Oheimb Gv. 2016. Crown and leaf traits as predictors

Lui Y, Dawson W, Prati D, Haeuser E, Feng Y, van Kleuenen M. 2016. Does greater Specific Leaf area plasticity help plants to maintain a high performance when shaded? Annals of Botany 118:1329-1336, Doi:10. 1093/aob/ mcw 180.

Mandák B, Krak K, Vít P, Pavlíkova Z, Lomonosova MN, Habibi F, Wang L, Jellen EN, Douda J. 2016. How genome size variation is linked with evolution within *Chenopodium sensu* Lato. Perspectives in plant Ecology, evolutions and systematic. 23(2016) 18-32.

Mandák B, Trávniček P, Paštova L, Kařinkova D. 2012. Is hybridization involved in the evolution of Chenopodium album aggregate? An analysis based on chromosome counts and genome size estimation. Flora 207 (2012) 530-540.

Mechant E, DeMarez T, Hermann O, Olson R, Bulcke R. 2008. Target site resistance to metamitron in *Chenopodium album* L. Journal of plant diseases and protection. Special issue XXI, 37-40.

Mehra PN, Malik CP. 1963. Cytology of some Indian Chenopodiaceae. International Journal of cytology, Cytosystematics and cytogenetics. Caryologia, 16:1 67-84, Doi:10.1080/0087114.1963. 10796085.

Mosyakin SL.2016.First record of *Chenopodium ficifollium* subsp.Blomiamianum (Chenopodiaceae) in North America phytoneuron 2016-33:1-6

of subtropical tree sapling growth rates. Journal of plant ecology. Volume 10. Number 1. Page 136-145.

Oguchi R, Ozaki H, Hanada K, Hikosaka K.2015. Which plant trait explains the variations in the relative growth rate and its response to elevated carbon dioxide concentration among *Arabidopsis thaliana* ecotypes derived from a variety of habitats? Oecologia (2016) 180:865-876. Doi 10.10/1007/s00442-015-3479-Z.

Ohri D.2015. The taxonomic riddle of Chenopodium album L.complex (Amaranthaceae).doi 10.1007/513237-015-0143-2.India.

Osone Y, Ishida A, Tateno M. 2008. Correlation between relative growth rate and specific leaf area requires associations of specific leaf area with Nitrogen absorption rate of roots. New Phytologist, 179:417-427. doi:10.1111/j. 1469-8137.2008.02476.x

Pal A, Banerjee B, Banerjee T, Marish M, Pal K. 2011. Hepatoprotective activity of *Chenopodium album* Linn. Plant against Paracetamol induced Hepatic injury in

rats. International journal of Pharmacy and Pharmaceutical sciences. ISSN.0975-1491.Vol3 suppl3.

Pommerening A, Muszta A. 2005. Methods of modelling Relative growth rate. Forest ecosystems (2011) 215. Doi 101186/s40663-015-0029-4

Pommerening and Muszata. 2015. Methods of modelling Relative growth rate. Forest ecosystems. Do 1 10.11861 540663-015

Poorter A, Remkes C, Lambers C. 1990. Carbon and nitrogen economy of 24 wild species differing in RGR. Plant physiology 94, 621-627. The Netherlands.

Poorter H, Garnier E. 1996. Plant growth analysis: An evaluation of experimental design and computational methods. Journal of experimental Botany. Vol.47, No.302, pp. 1343-1351. The Netherlands.

Poorter H, Remkes C, 1990. Leaf area ratio and Net assimilation rate of 24 wild species differing in Relative growth rate. Oecologia (1990) 83:553-559. The Netherlands.

Poorter H, Van Rijin CPE, Vanhala TK, Verhoeven KJF, deJong YEM, Stam P, Lambers H.2005. A genentic analysis of Relative growth rate and underlying components in Hordeum spontaneum. Ecophysiology. doi:10.1007/s.00442-004-1705-1.Oecologia(2005) 142:360-377.

Puglieli G, Spoletini A, Fabrini G, Gratani L. 2017.Temperature responsiveness of seedlings maximum Relative growth rate in three Mediterranean Cistus species. Journal of plant Ecology: Volume 10, Number 2, pages 331-339, doi:10.1093/jpe/rtw028.

Rahiminejad and Gornall. 2004. Flavoid evidence for alloploidy in the *Chenopodium album* aggregate(Amaranthaceae). Plant systematics and evolution. Doi 10.1007/s00606-003-0108-9

Rahman A,James T, Trolove M. (2014).Characteristics and control of dicamba-resistant common Lambsquarters (Chenopodium album). Weed Biology and Management 14, 88-89(2014). Newzealand.

Ramirez-Valiente JA, Center A, Sparks JP, Sparks KL, Etterson JR, Longwell T, Pliz G, Ramsey J, Ramsey TS. 2014. Ecological studies of polyploidy in the 100 years following its discovery. Phil. Trans. R.Soc. 369. 20130352. http://do.doi: org/10.1098/rstb.2013.0352

Ranjbar MRR.1995. Taxonomy and biosystematics of the *Chenopodium album* aggregate. Phd thesis. Department of Botany. University of Leicester.

Ravindhranath K, Babu AN, Mohan GVK. 2016. Removal of Chromium (VI) from polluted waters using absorbents derived from *Chenopodium album* and Eclipta Prostrate plant materials. International journal of Chem Tech research. Vol.9. No.03 pp 506-516.

Rees M, Osborne CP, Woodward FI, Hulme SP, Turnbull LA, Taylor SH. 2010. Partitioning the components of RGR: How important is plant size variation? The American Naruralist 176, No.6

Rutherfold S, Bonser SP, Wilson PG, Rossetto M. 2017. Seedling response to environmental variability: The relationship between phenotypic plasticity and

evolutionary history in closely related Eucalyptus species: American journal of Botany 104(6): 840-857. Sydney. Australia.

Santos FT, Ludwig F, Costa LAM, Costa MSSM, Remor MB, Silva PER. 2016. Growth analysis of potted gerbera conducted with mineral fertilization and organic fertigation. Cien. Inv.Agr. 43(1):111-120. Ornamental crops.

Scheiss J, Bruyn L. 2004. Excess of nutrients results in plant stress and decreased grass miner performance. Entamologia Experimentalis et Applicata, Winnie Lestruat 25, 1070 Brussels, Belgium.

Shipley B. 2017. Trade-offs between net assimilation rate and specific leaf area in determining relative growth rate: Relationship with daily irradiance. Functional ecology 2002.16,000-000. Canada J1K ZR1.

Shipley B.2006. Net assimilation rate, Specific leaf area and leaf mass ratio: Which is most closely related with Relative growth rate? A meta- analysis. Functional ecology 20, 565-574. Canada

Singh S. 2010. Understanding the weedy Chenopodium in the North Central States. Urbana.

Solymosi P, Lehoczki E, Laskay G. 1986. Difference in herbicide resistance to various Taxonomic populations of common Lambsquarters (*Chenopodium album)* and late-Flowering Goosefoot (*Chenopodium strictum)* in Hungary. Weed sience. Vol.34, No.2.(March, 1986).pp.175-180.

South DB. 1995.Relative growth rates: a critique south African forestry. Journal 173:43-48.

Sugiyama S. 2005. Relative contribution of meristem activities and specific leaf area to shoot relative growth rate in C3 grass species. Functional Ecology. 19, 925-931. Doi:10.1111/j.1365-2435. 2005-01043.X.

Tanveer M, Shah AN.2007. An insight into a salt stress tolerance mechanisms of chenopodium album. Environ Sci Pollut .Res(2017) 24:16531-16535.

Tessmer OL, Yuhua J, Cruz JA, Kramer DM, Chen J. 2013. Functional approach to high throughput plant growth analysis. BMC Systems Biology. Singapore.

Tomayo-Ordóñez MC, Espinosa –Barrera LA, Tomayo- Ordóñez YJ, Ayil-Gutiérrez B.SanchezTeyer LF.2016.Advances and perspectives in the generation of polyploid plant and species. Euphytica. Doi 10:1007/s10681-016-1646-X.University of Illinois at Urbana- Champaign.

Van Laere K, França SC, Vansteenkieste H, van Huylenbrorck J, Steppe K, Van Labeke MC.2010. Influence of ploidy level on morphology, growth and drought susceptibility in Spathiphyllum wallisii. Acta Physiol plant(2011) 33:1149-1156.001 10.1007/s11738-010-0643-2

Vega-Galvez A. Miranda M, Vergara J, Uribe E, Puente L, Martinez EA. 2010. Nutritional facts and functional potential of Quinoa (Chenopodium quinoa Wild) an ancient Andean grain. A review. Journal of the science of food and agriculture.doi:10.1002/jsfa.4158.

Villar R, Marañón T,Quero JL, Panadero P, Arenas F, Lambers H.2005. Variation in relative growth rate of 20 Aegilops species (Poaceae) in the field. The importance

of net assimilation rate or specific leaf area depends on the time scale. Plant and soil (2005) 272:11-27. Doi 10.1007/s11104-004-3846-8.

Villas JS, Pannell JR. 2017. No differences in plasticity between ploidy levels in the Mediterranean herb Mercuriaslis annua. Scientific reports. 001.10.1038.

Vít P, Krak K, Trávniček P. Douda J, Lomonosova MN, Mandák B.2016.Genome size stability across Eurasian *Chenopodium* species (Amaranthaceae). Botanical Journal of the Linnean society, 637-649.

Walsh BM, Adhikary D, Maughan PJ, Emshwiller E, Jellen EN.2015. Chenopodium polyploidy inferences from salt overly sensitive 1(SOS1) Data 1. American journal of Botany.

Wang J, Lan X, Jiang S, Ma Y, Zhang S, Li Y, Li X, Lan H. 2017. CaMKK1 from *Chenopodium* positively regulates salt and drought tolerance in transgenic tobacco. Plant cell tiss organ cult(2017). 130:209-225. Doi 10:1007/511240-017-1216-5

Wang P, Weiner J. Cahill JF, Zhou DW, Bian. HF, Song YT, Sheng LX. 2014.Shoot Competition, root competition and reproductive allocation in *Chenopodium acuminatum.* Journal of ecology 182, 1696. Doi:10.1111/1365-2745.12313.

Wang Q. Liu H, Gao A, Yang X, Liu W, Li X, Li L. 2012. Intergenomic rearrangements after polyploidization of Kengyilia thoroldiana (Poaceae:Triticeae) Affected by environmental factors. PLOS ONE 7(2): e31033.doi: 10.137/Journal.Pone.0031033.

Williams JT,1963.*Chenopodium album* L. 1963. Journal of Ecology . Vol.51. No. 3(pp.711-725). British ecology society.

Wilson HD. 1980. Artificial Hybridization among species of *Chenopodium* sect. *Chenopodiu*. Systematic Botany. America society of plant taxonomists.

Xie Y, Zhu X. Ma Y, Zhao J, Li L, Li Q. 2017. Natural hybridization and reproduction isolation between two Primula species. Journal of intergrative biology. doi:10:1111/jipb.12546.China.

Yadav N, Vasudeva N, Singh S, Sharma SK. 2006. Medicinal properties of Chenopodium Linn. Natural products Radiance, Vol.6(2), 2007.Pp.131-134.

Yerka MK, de Leon N, Stoltenberg DE. 2012. Pollen-Mediated gene flow in common lambsquaters (*Chenopodium album)* Bio one Research evolved. Weed sciences society of America. 60:600-606.

Zhang G, Johnkan M, Hohjo M, Tsukagoshi S, Maruo T. 2017. Plant growth and photosynthesis response to low potassium conditions in three Lettuce (Lactusa sativa) types. The horticulture Journal 86(2): 229-237.2017.doi:10.2603/hortJob008.

APPENDICES

List of Figures

List of tables