

Clonal Integration in *Ochthochloa Compressa* under Extreme  
Environmental Conditions



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This dissertation is submitted for the degree of  
Doctor of Philosophy

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Februarys 2018

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## My thanks and appreciation

To my parents,  
my wife (Um Fajr) and my kids,  
my brothers and sisters,  
whose made all of this possible,  
for their endless encouragement and patience.

## Abstract

While multiple studies have indicated the benefit of clonal integration and its role in resisting harsh environmental conditions, many researchers have indicated the need for further studies to understand fully the role of clonal integration and what determines the optimal strategies in various environments. In this project a series of studies were carried out in an extremely arid area (Al Thumam area in the Arabian Peninsula) to contribute to the knowledge of the benefits of clonal integration and understanding the behaviour of the clonal grass *Ochthochloa compressa*. This study is unique because it has investigated the benefits of clonality in one of the harshest environments where clonal plants are found.

Experiment 1. I aimed to understand the patterns of spread and expansion of stolons, particularly whether stolons grew in random directions or are directed to better patches by the mother ramets to establish new daughter ramets maximizing their chances of success. I measured nutrient contents (N, P and K) in patches where mother plants grew, and where daughter ramets had established. In addition, I sampled nearby unoccupied patches. Mother plants were found in patches with higher N concentrations than where the daughter ramets were found. There were no differences in concentration of P; while K was the lowest where not fully rooted daughters were found. The results suggest that daughter ramets did not establish in the best areas, indicating that the spread and expansion of stolons in the *O. compressa* occurs randomly.

Experiment 2. I investigated the effects of the addition of fertilizer to mother and daughter ramets, including addition of nutrients to daughters disconnected from the mother ramets. Cutting the stolons caused to death of the daughter because these ramets were still dependent on the mother ramets. When connected, mothers that received nutrients affected some transference nutrients to daughter ramets. In contrast, the

daughters accumulated the nutrients in above ground tissue when receiving added fertilizer, and there was no sign of transference to the mother ramet. Nutrient addition did not affect in any case the efficiency photosynthetic in both mother and daughter ramets.

Experiment 3. In this experiment, I focused on the effect of the distance between mother and daughter ramets on the performance of daughter ramets. Daughters located close to the mother ramets could suffer competition by the mother if they are within the area of the root system. The results showed no significant differences between mother and daughter ramets in the concentration of nitrogen, phosphorus, and potassium nor in photosynthetic activity. This indicates that the daughters have the ability to resist competition through continued support from their mothers despite the scarcity of resources and the harsh environmental conditions in the study area.

Experiment 4. In this experiment, I studied the effect of simulated grazing on both mother and daughter ramets when one of them was clipped and 50-60% of the leaves were removed, while remaining connected. Clipping did not affect the N content of mother ramets, but the concentration of phosphorus was decreased by clipping. K was lower in mother ramets connected to clipped daughters. Daughters connected to clipped mothers had higher N concentration but K and P did not change. Clipping of daughters did not have any effect on mother ramets concentration of nutrients. Photosynthetic efficiency did not record any significant differences when ramets were clipped. The results indicate that *O. compressa* strategy to resist grazing consists mainly in continuing to support daughter ramets.

As far as I know, this is the first study of the phenomenon of clonal integration for *O. compressa*. This study revealed the importance of clonal integration for *O. compressa* to resistance of the harsh environmental conditions. Under the harsh



conditions these plants live, the preferred strategy seems to be for the mother ramets to expand by producing ramets in random sites and heavily subsidize their growth with nutrient, and almost certainly water. Indeed, I documented strong evidence of transfer of nutrients through the stolons from mother ramets to daughter ramets but no evidence of transfer in the other direction even when nutrients were supplied to daughters. Further, seemingly surplus N available to the mother when clipping limited their foliar tissues was directed to daughters rather than to re-sprouting (which was probably limited by water availability).

The insights obtained on the biology of *O. compressa* are critical as it is a native plant in a harsh environment, and it is suitable for fodder for pastoral animals, as well as having potential for restoration of degraded areas. Further, it provides new insights into the phenomenon of clonal integration in harsh habitats, and area which still needs further study and research.

## Declaration

I hereby declare that except where specific reference is made to the work of others, the contents of this dissertation are original and have not been submitted in whole or in part for consideration for any other degree or qualification in this, or any other university. This dissertation is my own work and contains nothing which is the outcome of work done in collaboration with others, except as specified in the text and Acknowledgements.

Mobarak Al Mosallam  
Februarys 2018

## Acknowledgements

During my PhD journey, I spent a great time at the University of Adelaide with the school, staff and students. I would like to thank all who made my journey a smooth and nice graduate school experience. Firstly, I would like to thank and show my appreciation to my supervisor Associate Professor José M. Facelli. He is the best enhanced to success in me and I still learning from him. Also, he taught me how to be part of the research community, how to be a good researcher and supervisor in future, and how to practise my academic career.

I would to thank our lab group to continuous support, advice and motivation. Also, I would like to thank anonymous examiners for their valuable comments and feedbacks on earlier drafts.

I owe of gratitude to my parents, my lovely wife Haya Bin Selleh (Um Fajr), my brothers and sisters for their constant encouragement and patience. They have been always with me at all time.

Finally, I express my sincere appreciation to King Abdulaziz City for Science and Technology (KACST), Saudi Arabia, who awarded me this scholarship to obtain on PhD degree.

## Chapter 1

# Clonal Integration in *Ochtochloa Compressa* Ecotypes under Various Environmental Conditions.

## Introduction

The clonal integration phenomenon is one of the mechanisms of asexual reproduction, where the clonal plants reproduce themselves through the production of ramets, which are genetically identical to the mother ramets (Yan *et al.*, 2013), and connected to the mother ramets by rhizomes or stolons (Stuefer *et al.*, 1994). The aims of the clonal integration mechanism through connection between mother and ramets are exporting resources and nutrients to daughter ramets from mother ramets by the stolon (Wolfer and Straile, 2012). Also, the integration mechanism contributes to the spread of stolons in harsh environments and open spaces with scarce resources (Dong and Alaten, 1999). One of the most important advantages of clonal plants is the ability to share nutrients, carbohydrates and water between mother and daughter ramets through stolons (Alpert and Mooney, 1986; Alpert, 1999; Pennings and Callaway, 2000; Peltzer, 2002; Yu *et al.*, 2004; Wang *et al.*, 2008; Liu *et al.*, 2009; Yu *et al.*, 2010; Xiao *et al.*, 2011; Martina and Von Ende, 2013; Yan *et al.*, 2013; Luo and Zhao, 2015), manipulating the composition of clonal plant communities (de Kroon and Knops, 1990; Xiao *et al.*, 2011) and explore open spaces unlike non clonal plants (Martina and Von Ende, 2013).

Besides, sharing resources in clonal integration mechanism can enhance the chance of survival and growth through resource sharing mechanism (Peltzer, 2002; Xiao *et al.*, 2010), and resistance to harsh environmental conditions (Wang *et al.*, 2008; Xiao *et al.*, 2010; Yu *et al.*, 2010) such as drought, stress (Stuefer *et al.*, 1994) grazing (Pennings and Callaway, 2000; Xiao *et al.*, 2010) and wind erosion (Yu *et al.*, 2002). It also contributes

to adapt to different environments (de Kroon and Knops, 1990) that through assist mother to a new ramets by nutrients and as be a source of resources for it. It should be noted that the behaviour of clonal plants varies according to plant species and type of environment (de Kroon and Knops, 1990; He *et al.*, 2011), hence the phenomenon of clonal integration needs further research and study to understand the role of the clonal integration mechanism in the spread of clonal plant stolons in harsh environments and their resistance to harsh environmental factors such as grazing and competition and drought.

Therefore, there is a great benefit in exploring different possibilities in clonal integration of the perennial grass *Ochthochloa compressa*, which is believed to possess all the traits for multipurpose use fir vegetation management. Indeed, such clonal traits can be efficient for combating desertification and rehabilitating of degraded rangelands.

## Literature Review

Clonal plants often consist of many connected individual ramets, which allows them to share resources via clonal integration. This may improve the ability of the plants to tolerate abiotic stress and play a crucial role in regulating their shoot growth (Wolfer and Straile, 2012). Clonal integration enables clonal plants to share nutrients, water and carbohydrates (Alpert, 1996; Wijesinghe and Hutchings, 1997) utilize efficiently patchy soil resources (Evans and Cain, 1995; Brewer and Bertness, 1996), and tolerate environmental stresses (Slade and Hutchings, 1987; Pennings and Callaway, 2000). Yu *et al.* (2008) suggested that erosion as well as severing of rhizomes has a negative impact on the number of ramets and it may result in a decrease in the number of ramets, leaves or biomass of the plants. This study further substantiates the findings of Yu *et al.* (2008) that the negative impacts of erosion mainly derives from the lack of protection of an adequately thick layer of sand, which causes fast dehydration of roots and rhizomes, thus precluding them from acquiring and transporting water and other essential resources. A

study by Zhao *et al.* (2011) proved that plants that have been buried in moderate amounts of sand can benefit of it by protecting the roots from drying and increased nutrient availability, to expand the area of the spread of the roots, which helps to increase the growth of shoots, roots, leaves and increase biomass as well (Zhao *et al.*, 2011). According to Yu *et al.* (2008), the numbers of leaves and the biomass increased by 37% in the case of moderate burial. However, the numbers of leaves and biomass dropped to 47% in the case of deep burial (Yu *et al.*, 2008). Therefore, the results of the study indicate that rhizome connections or clonal integration considerably enhanced the ability of plants to cope with drought (Yu *et al.*, 2008).

Slade and Hutchings (1987) conducted a study to determine the costs and benefits of the physiological integration between the ramets in the clonal perennial herb *Glechoma hederacea*. They found that the number of leaves, biomass, number of ramets and stolon expansion becomes significant in the determination of the level of integration because the movement of resources within the stolons is highly biased in an acropetal direction. The authors contend that clonal integration thus leads to reduce the effects of adverse local conditions that may hamper the growth and survival of plants in specific patches. The study also points to the importance of ramet age as younger ones, irrespective of growing conditions, are capable of producing two primary stolons but older ramets required nutrient-rich conditions to achieve the same productivity (Slade and Hutchings, 1987). Another study by Wulff (1991) supports the contention that clonal integration, through maintenance of subsidies among ramets that can achieve larger size, helps the growth and persistence of clonal plants. In their study on clonal integration and the effects of herbivory in old-field perennials, Schmid *et al.* (1988) compared the effects of defoliation on shoot performance in connected shoots and severed rhizomes. They tested the hypothesis that the impact of defoliation will be stronger in the most clonally

integrated than in less clonally integrated plants (Schmid *et al.*, 1988). Their findings suggested that the removal of about a half of the foliage from shoots produced varying impacts on them, depending on the species, the density of shoots but largely on the rhizome integrity through the connections between shoots (Schmid *et al.*, 1988). Thus, this study also corroborates that clonal integration can be highly beneficial to plants as it assists their growth and survival in adverse circumstances.

Amsberry *et al.* (2000) found that clonal integration enhanced the survival of *Phragmites australis* under salinity conditions, especially in low areas. Furthermore, they noted that the vegetative reproduction and tolerance to adverse environmental conditions were increased by clonal integration. In the marshland grass *Spartina alterniflora*, Xiao *et al.* (2010) found that clonal integration of the daughter ramets considerably enhances their flood tolerance. The basic premise of their study was that since clonal plants possess the ability to share water as well as other resources through vascular connections between ramets, clonal integration increases the ability of individual ramets to grow and survive even in adverse circumstances (Alpert, 1996 and 1999; Xiao *et al.*, 2010). Mauchamp *et al.* (2001) and Xiao *et al.* (2010) proved that clonal integration play an important role in stressful environments and that daughter ramets connected with mother ramets extend much more quickly than severed ones. Thus, it is clear that clonal integration allows mother ramets to pass on resources to daughter ramets so as to enable them to escape quickly from stress, such as submergence (Xiao *et al.*, 2010).

Similarly, Yu *et al.* (2002) found that clonal integration enhances the ability of *Potentilla anserina* to respond and survive to partial sand burial. The experiment in this study was conducted in the semi-arid and desertified area of Ordos Plateau of China, which is exposed to frequent strong winds. The results suggest that the biomass, number of ramets and leaves as well as the leaf area markedly decreased during the burial treatments when

stolon connections were cut (Yu *et al.*, 2002). However, connected ramets enabled plants to resist frequent sand transport by wind, demonstrating the benefit of clonal integration (Yu *et al.*, 2002).

The main reason for this plant strategy is that in clonally integrated species the mother ramets can facilitate a seamless flow of resources, thereby supporting the growth of their daughter ramets making them capable of withstanding adverse conditions. Caraco and Kelly (1991) found that anatomical connection in plants is key to physiological integration, through transfer of resources from mother ramets to daughter ramets. However, when resources are limited, such transfer can reduce the growth of mother ramets while it increases the daughter's growth.

Through the clonal integration mechanism, the mother ramets are providing the nutrient for daughters through the stolons (Wolfer and Straile, 2012), while there is different levels of benefit from the clonal integration which depends on the plant species and pattern of habitats (Alpert and Mooney, 1986). The mother ramets are the main source of nutrients for their daughter ramets (Alpert, 1999). Under harsh environmental condition the mother's ramets can continue to support daughter ramets for years (Slade and Hutchings, 1987; Yu *et al.*, 2004), until daughter ramets are able to sustain themselves. In harsh environments this may require larger size of the daughter ramets to reach independence. As growth is slow in those conditions, the period of dependence of daughters may be much longer in harsh environments (Alpert and Mooney, 1986) to reduce the environmental pressures on the ramets (Alpert and Mooney, 1986; Pennings and Callaway, 2000). However, this implies a high cost to the mother ramets, and it can be expected that under extreme conditions the cost of the integration to the mother plant can be less than the benefits obtained by the genet by supporting the daughter ramets



(Slade and Hutchings, 1987). This in theory should limit the distribution of clonal plants into extreme environments.

In a study of the ecological role of clonal integration of *Psammochloa villosa* to withstand sand accumulation (Yu *et al.*, 2004) it was found that increasing burial depths considerably decreased the survival of the ramets. The authors further contended that clonal integration allows *P. villosa* to survive and re-establish their population and form colonies in new bare grounds through fast expansion of rhizomes (Liu *et al.*, 2009). This is possible mainly because clonal integration can enhance the ability of the plant to elongate its vertical structures and stay above accumulating sand with the help of resources transported through the ramets that remain unburied (Yu *et al.*, 2004). Chen, Lei and Dong (2010) documented that the effects of sand burial on clonal integration varies according to the depth of burial, that when the burial depth are increasing will decreasing the resistance of clonal integration for that. Additionally, the clonal plants have the ability to transport essential resources through the interconnected ramets via source-sink regulation. Clonal integration also ameliorates the negative impacts of sand accumulation on survival and growth of *Carex preclear* (Shi *et al.*, 2004; Chen *et al.*, 2010). Thus, they argue that clonal plants can be utilised as a source for stabilising sand while re-vegetating desertified regions (Chen *et al.*, 2010).

Clonal integration of plants has been also found to have benefits in tropical conditions such as evergreen forests, as shown by the results of a study conducted by Du *et al.* (2010). The researchers refer to evidence from various studies that show that while clonal integration increases the performance of plants, the benefits depend on the ability of the plant to uptake resource or produce photosynthesis (Du *et al.*, 2010).

Stuefer (1996) and Chen *et al.* (2004) suggested that the resources for plants are patchily distributed in space and time even at scales relevant to individuals. In this context, Alpert

(1999) highlighted the importance of studying clonal growth in habitats with different patterns of resource availability to understand the role of clonal integration in ecological as well as evolutionary terms. The results of their studies indicated that morphological plasticity does not aid the access of resources, when supplied in an unpredictable manner. So, under such situations clonal growth depend on access to resources rather than the clonal morphology.

In his study of the effects of clonal integration on plant plasticity in *Fragaria chiloensis*, Alpert (1999) argues that since clonal plants can transport resources among ramets growing in different microsites, they can also change the plastic responses of ramets depending on environmental conditions.

Several plant species have some degree of clonal growth that have a crucial role in preserving ecosystem functions and community assemblage (Wilsey, 2002; Yu *et al.*, 2010; Xiao *et al.*, 2011). However, their study concludes that clonal integration has very little effect on plant diversity in dunes plant communities, but they attributed this finding to the unforeseen high mortality rate of some species and hence emphasise the need for further studies on such topic (Yu *et al.*, 2010). It has been found that without clonal integration, under high salinity conditions the youngest ramets of *Spartina alterniflora* died, which suggests that clonal integration is more important for young ramets (Alpert and Mooney, 1986; Xiao *et al.*, 2011). They further argue that since young plants are at higher risk from environmental factors, clonal integration is crucial for their growth and survival.

Sand burial and accumulation change abiotic and biotic conditions of plants and thereby triggers changes in their physiology and morphology and thus affect their growth and chances of survival (Maun, 1998; Yu *et al.*, 2002). However, clonal integration can help mitigate this problem, if the stolon connection between the buried and unburied ramets is

maintained as evidenced through the experiments run by Yu *et al.* (2002). The unburied ramets will be able to provide the resources to the buried ramets through clonal integration and thus it allows the adaptation of the plant to conditions where sand accumulation is pervasive. In their study relating to plant growth and foraging for a patchy resource, Oborny and Englert (2012) found that clonal integration helps plants to regulate their growth pattern to the type of resources available in their environment (Oborny and Englert, 2012). They also found that phenotypic plasticity of growth as a critical factor for understanding the behaviour of individual plants in adapting to the environment (Silvertown and Gordon, 1989; Novoplansky, 2002; Oborny and Englert, 2012). The main advantage of clonal plants in this regard is that each ramet has access to resources in the environment depending on the area covered by roots and foliage (Oborny and Englert, 2012). Thus, even if some ramets are not able to extract resources, the resources obtained by other ramets get distributed through the integrated clonal system. Thus, when plants forage into a different environment, clonal integration will help them to adapt to the new habitat as clones have resource sharing capabilities.

Xiao, Yu, Wang and Han (2011) documented that physiological integration of plants can help them invade heterogeneous habitats or escape from their current environment. Thus, due to the benefits deriving from clonal integration plants inhabiting coastal areas can migrate into new areas when sand accumulated and effectively adapt to the changed environment and survive there. This study offers evidence to suggest that clonal integration is highly significant for the “survival and tolerance” of ramets in stressful and adverse environments (Xiao *et al.*, 2011). The authors further argue that during the period of the experiment, the plants displayed a strong potential for acropetal stolon growth resulting in the production of ample biomass and offspring ramets, when clonally integrated (Xiao *et al.*, 2011). On the other hand, severing the stolon resulted in a decrease

in daughter ramets, while mother ramets continued to grow at the expense of progeny ramets (Xiao *et al.*, 2011). The study further shows that when plants forage into bare habitats such as newly-formed sand dunes from nearby vegetated environments such as coastal belts, they place more ramets into exposed habitats, suggesting that mother plant can direct somehow the place new daughter ramet establish. Further their results prove the contention that connection to a mother plant in a more suitable environment allow the new ramets to escape from harmful environment as they adapt to it through plasticity or even modify the environment by accumulating finer particles (Macek and Lepš, 2003; Sampaio *et al.*, 2004; Xiao *et al.*, 2011).

In addition, studies of the effects of clonal integration on plant plasticity, predicts that clonal integration will decrease the need for plasticity in plants because it equalizes concentration of resources among ramets (Alpert, 1999). The primary objective of Alpert (1999) study has been testing the additional manners in which clonal integration modifies the plasticity of plants under different levels of morphological organisation (Alpert 1999). Among other findings, he found evidence that clonal connection induced a range of plastic responses to light and nitrogen that reversed the plastic response occurring in single ramets (Alpert 1999). The author concluded that the plasticity facilitated by clonal integration enables ramets to respond to conditions in a single microsite or those in a set of multiple sites and the resultant differences (Alpert 1999). Thus, it can be construed that clonal integration provides resistance to plants to adapt to the varying conditions in a new habitat and facilitates their growth and survival. Thus, it transpires that clonal integration enhances the ability of the plant to resist the adverse conditions in the newly formed sand accumulation on beaches.

In spite of the seemingly large number of studies presented above, the information about clonal integration and clonal plants behaviour is still insufficient and needs further study

and investigation. In order to contribute to the understanding of this mechanism, I seek to understand the behaviour of the integration of *Ochthochloa compressa*. This is the first study of *O. compressa* in the Arabian Peninsula. In order to contribute to the understanding of the mechanism of clonal integration, I attempt to document the behaviour of this clonal plant which is found throughout the Arabian Peninsula and extends from northern Africa to western Indian subcontinent. The insights obtained on the biology of *O. compressa* are critical as it is a native plant in a harsh environment, and it is suitable for fodder for pastoral animals, as well as having potential for restoration of degraded areas.

Grazing, nutrient availability and interactions between mother and daughter ramets are factors that strongly affect plants in dry environments. In our research, I am focused on studying the relationship between soil and mother ramets and role of abundance of nutrients in soil in spread and establishment of new daughter ramets, through analyzing the concentration of nitrogen, phosphorus and potassium in soil and both mother and daughter ramets. Also, I studied the effect of nutrient abundance, competition and grazing on the concentration of nitrogen, potassium and phosphorus in both mother and daughter ramets when connected and disconnected with mother ramets as an indicator of the health and effect of it. Furthermore, because the remoteness of the University of Adelaide precluded long term studies, I measured the efficiency of photosynthesis in both mother and daughter ramets to determine the effect of that environmental conditions on photosynthesis and hence potential growth to document the behaviour of mother and daughter ramets when exposed to extreme environmental conditions.

## Research Aims

- To investigate the mechanism of spread of stolons and the establishment of new ramets in *Ochthochloa compressa*, is it ecological strategy by mother ramets whether depend on the abundance of nutrients in the soil or no?
- To test for evidence of nutrient movement between mother and daughter ramets.
- To determine the effect of harsh environment (such as neighbouring plants) on the mother's ability to spread and establishing a new ramets.
- To examine the effect of clipping by 50 - 60% on both mother and daughter ramets.

## References

- Alpert, P & Mooney, H** 1986. 'Resource sharing among ramets in the clonal herb, *Fragaria chiloensis*', *Oecologia*, vol. 70, no. 2, pp. 227-233.
- Alpert, P** 1996, 'Nutrient sharing in natural clonal fragments of *Fragaria chiloensis*', *Journal of Ecology*, pp. 395-406.
- Alpert, P** 1999, 'Clonal integration in *Fragaria chiloensis* differs between populations: ramets from grassland are selfish', *Oecologia*, vol. 120, no. 1, pp. 69-76.
- Alpert, P** 1999, 'Effects of clonal integration on plant plasticity in *Fragaria chiloensis*', *Plant Ecology*, vol. 141, no. 1-2, pp. 99-106.
- Amsberry, L, Baker, M.A, Ewanchuk, P.J & Bertness, M.D** 2000, 'Clonal integration and the expansion of *Phragmites australis*', *Ecological applications*, vol. 10, no. 4, pp. 1110-1118.
- Brewer, J.S & Bertness, M.D** 1996, 'Disturbance and intraspecific variation in the clonal morphology of salt marsh perennials', *Oikos*, pp. 107-116.

- Caraco, T & Kelly, C.K** 1991, 'On the Adaptive Value of Physiological Integraton in Colonal Plants', *Ecology*, pp. 81-93.
- Chen, J-S, Lei, N-F & Dong, M** 2010, 'Clonal integration improves the tolerance of *Carex praeclara* to sand burial by compensatory response', *Acta Oecologica*, vol. 36, no. 1, pp. 23-28.
- Chen, J-S, Yu, D, Liu, Q & Dong, M** 2004, 'Clonal integration of the stoloniferous herb *Fragaria vesca* from different altitudes in Southwest China', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 199, no. 4, pp. 342-350.
- de Kroon, H & Knops, J** 1990. 'Habitat exploration through morphological plasticity in two chalk grassland perennials', *Oikos*, pp. 39-49.
- Dong, M & Alaten, B** 1999. 'Clonal plasticity in response to rhizome severing and heterogeneous resource supply in the rhizomatous grass *Psammochloa villosa* in an Inner Mongolian dune, China', *Plant Ecology*, vol. 141, no. 1-2, pp. 53-58.
- Du, J, Wang, N, Alpert, P, Yu, M-J, Yu, F-H & Dong, M** 2010, 'Clonal integration increases performance of ramets of the fern *Diplazium glaucum* in an evergreen forest in southeastern China', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 205, no. 6, pp. 399-403.
- Evans, JP & Cain, ML** 1995, 'A spatially explicit test of foraging behavior in a clonal plant', *Ecology*, vol. 76, no. 4, pp. 1147-1155.
- He, WM, Alpert, P, Yu, FH, Zhang, LL & Dong, M** 2011. 'Reciprocal and coincident patchiness of multiple resources differentially affect benefits of clonal integration in two perennial plants', *Journal of Ecology*, vol. 99, no. 5, pp. 1202-1210.
- Liu, H-D, Yu, F-H, He, W-M, Chu, Y & Dong, M** 2009, 'Clonal integration improves compensatory growth in heavily grazed ramet populations of two inland-dune grasses',

- Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 204, no. 4, pp. 298-305.
- Luo, W & Zhao, W** 2015. 'Effects of wind erosion and sand burial on growth and reproduction of a clonal shrub', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 217, pp. 164-169.
- Macek, P & Lepš, J** 2003, 'The effect of environmental heterogeneity on clonal behaviour of *Prunella vulgaris* L', *Plant Ecology*, vol. 168, no. 1, pp. 31-43.
- Martina, J & Von Ende, C** 2013. 'Increased spatial dominance in high nitrogen, saturated soil due to clonal architecture plasticity of the invasive wetland plant, *Phalaris arundinacea*', *Plant Ecology*, vol. 214, no. 12, pp. 1443-1453.
- Mauchamp, A, Blanch, S & Grillas, P** 2001, 'Effects of submergence on the growth of *Phragmites australis* seedlings', *Aquatic Botany*, vol. 69, no. 2, pp. 147-164.
- Maun, M** 1998, 'Adaptations of plants to burial in coastal sand dunes', *Canadian Journal of Botany*, vol. 76, no. 5, pp. 713-738.
- Novoplansky, A** 2002, 'Developmental plasticity in plants: implications of non-cognitive behavior', *Evolutionary Ecology*, vol. 16, no. 3, pp. 177-188.
- Oborny, B & Englert, P** 2012, 'Plant growth and foraging for a patchy resource: A credit model', *Ecological Modelling*, vol. 234, pp. 20-30.
- Peltzer, DA** 2002. 'Does clonal integration improve competitive ability? A test using aspen (*Populus tremuloides* [Salicaceae]) invasion into prairie', *American journal of botany*, vol. 89, no. 3, pp. 494-499.
- Pennings, SC & Callaway, RM** 2000. 'The advantages of clonal integration under different ecological conditions: a community-wide test', *Ecology*, vol. 81, no. 3, pp. 709-716.



- Sampaio, M.C, Araújo, T.F, Scarano, F.R & Stuefer, J.F** 2004, 'Directional growth of a clonal bromeliad species in response to spatial habitat heterogeneity', *Evolutionary Ecology*, vol. 18, no. 5-6, pp. 429-442.
- Schmid, B, Puttick, G, Burgess, K & Bazzaz, F** 1988, 'Clonal integration and effects of simulated herbivory in old-field perennials', *Oecologia*, vol. 75, no. 3, pp. 465-471.
- Shi, L, Zhang, Z, Zhang, C & Zhang, J** 2004, 'Effects of sand burial on survival, growth, gas exchange and biomass allocation of *Ulmus pumila* seedlings in the Hunshandak Sandland, China', *Annals of Botany*, vol. 94, no. 4, pp. 553-560.
- Silvertown, J & Gordon, D.M** 1989, 'A framework for plant behavior', *Annual Review of Ecology and Systematics*, vol. 20, pp. 349-366.
- Slade, A & Hutchings, M** 1987, 'An analysis of the costs and benefits of physiological integration between ramets in the clonal perennial herb *Glechoma hederacea*', *Oecologia*, vol. 73, no. 3, pp. 425-431.
- Stuefer, J.F** 1996, 'Potential and limitations of current concepts regarding the response of clonal plants to environmental heterogeneity', *Vegetatio*, vol. 127, no. 1, pp. 55-70.
- Stuefer, JF, During, HJ & de Kroon, H** 1994. 'High benefits of clonal integration in two stoloniferous species, in response to heterogeneous light environments', *Journal of Ecology*, pp. 511-518.
- Wang, N, Yu, F-H, Li, P-X, He, W-M, Liu, F-H, Liu, J-M & Dong, M** 2008. 'Clonal integration affects growth, photosynthetic efficiency and biomass allocation, but not the competitive ability, of the alien invasive *Alternanthera philoxeroides* under severe stress', *Annals of Botany*, vol. 101, no. 5, pp. 671-678.
- Wijesinghe, DK & Hutchings, MJ** 1997, 'The effects of spatial scale of environmental heterogeneity on the growth of a clonal plant: an experimental study with *Glechoma hederacea*', *Journal of Ecology*, pp. 17-28.

- Wilsey, B** 2002, 'Clonal plants in a spatially heterogeneous environment: effects of integration on Serengeti grassland response to defoliation and urine-hits from grazing mammals', *Plant Ecology*, vol. 159, no. 1, pp. 15-22.
- Wolfer, SR & Straile, D** 2012. 'To share or not to share: clonal integration in a submerged macrophyte in response to light stress', *Hydrobiologia*, vol. 684, no. 1, pp. 261-269.
- Wulff, J.L** 1991, 'Asexual fragmentation, genotype success, and population dynamics of erect branching sponges', *Journal of Experimental Marine Biology and Ecology*, vol. 149, no. 2, pp. 227-247.
- Xiao, K, Yu, D, Wang, L & Han, Y** 2011, 'Physiological integration helps a clonal macrophyte spread into competitive environments and coexist with other species', *Aquatic Botany*, vol. 95, no. 4, pp. 249-253.
- Xiao, Y, Tang, J, Qing, H, Ouyang, Y, Zhao, Y, Zhou, C & An, S** 2010, 'Clonal integration enhances flood tolerance of *Spartina alterniflora* daughter ramets', *Aquatic Botany*, vol. 92, no. 1, pp. 9-13.
- Xiao, Y, Tang, J, Qing, H, Zhou, C & An, S** 2011, 'Effects of salinity and clonal integration on growth and sexual reproduction of the invasive grass *Spartina alterniflora*', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 206, no. 8, pp. 736-741.
- Yan, X, Wang, H, Wang, Q & Rudstam, LG** 2013. 'Risk spreading, habitat selection and division of biomass in a submerged clonal plant: Responses to heterogeneous copper pollution', *Environmental pollution*, vol. 174, pp. 114-120.
- Yu, F, Chen, Y & Dong, M** 2002, 'Clonal integration enhances survival and performance of *Potentilla anserina*, suffering from partial sand burial on Ordos plateau, China', *Evolutionary Ecology*, vol. 15, no. 4-6, pp. 303-318.

- Yu, F, Dong, M & Krüsi, B** 2004, 'Clonal integration helps *Psammochloa villosa* survive sand burial in an inland dune', *New Phytologist*, vol. 162, no. 3, pp. 697-704.
- Yu, F-H, Wang, N, He, W-M & Dong, M** 2010. 'Effects of clonal integration on species composition and biomass of sand dune communities', *Journal of Arid Environments*, vol. 74, no. 6, pp. 632-637.
- Yu, F-H, Wang, N, He, W-M, Chu, Y & Dong, M** 2008, 'Adaptation of rhizome connections in drylands: increasing tolerance of clones to wind erosion', *Annals of Botany*, vol. 102, no. 4, pp. 571-577.
- Zhao, H-L, Zhao, X-Y, Zhang, T-H & Zhou, R-L** 2011, 'Effects of wind blow and sand burial stress on seedling growth and photosynthetic properties of *Caragana microphylla*', *Energy Procedia*, vol. 11, pp. 2405-2412.

## Chapter 2

# Do mother ramets establish new ramets randomly or by mother ramets selecting better patches?

### Introduction

Drought, sand burial, and wind erosion are some of the most significant environmental stresses encountered by desert plants (Luo and Zhao, 2015). These stresses cause a decrease in water and soil resources, mainly due to poor soils (Wolfer and Straile, 2012). Under harsh conditions the heterogeneity of the environment, particularly nutrient patchiness, can determine plant distribution (Bartels and Chen, 2010; He *et al.*, 2011; Mommer *et al.*, 2011), productivity (Luo and Zhao, 2015) and biodiversity (Clark *et al.*, 2010; He *et al.*, 2011). Clonal plants can maximise the use of such environments either by averaging the resource in different patches or by “exploring” the area surrounding a mother plant by sending ramets in different direction and then supporting those in the best patches.

The phenomenon of clonal integration is widespread in dry areas and sandy environments as a mechanism that allows plants to adapt to the difficult environmental conditions (Salzman and Parker, 1985; Slade and Hutchings, 1987; de Kroon and Knops, 1990; Pennings and Callaway, 2000; Peltzer, 2002; D’Hertefeldt *et al.*, 2011; Wolfer and Straile, 2012). Indeed, this phenomenon increases the chances of survival and reduces the negative effects of environmental stresses (Peltzer, 2002; Yu *et al.*, 2008; Luo and Zhao, 2015; Martina and Von Ende, 2013; Evans, 1988; Brewer and Bertness, 1996) by sharing nutrients, water, and carbohydrate resources between mother and daughter ramets (Peltzer, 2002; D’Hertefeldt *et al.*, 2011; Yan *et al.*, 2013). To a large extent, this is done through the transfer of water, nutrients and carbohydrates from mother to daughter ramets

(Alpert and Mooney, 1986; Stuefer and Hutchings, 1994; Alpert, 1996; Wijesinghe and Hutchings, 1997; Peltzer, 2002).

The clonal integration mechanism allows clonal plants to replicate themselves asexually (Walfer and Straile, 2012), enabling them to affect the composition of plant communities (de Kroon and Knops, 1990). This mechanism also lets clonal plants explore the new habitats potentially modulating the maternal investment to suit the best strategy, unlike non-clonal plants which release seeds with similar maternal investment (Slade and Hutchings, 1987; Sutherland and Stillman, 1988; de Kroons and Hutchings, 1995; Chen *et al.*, 2010; Martina and Von Ende, 2013). Exploring the environment around the mother plant with ramet-producing stolons that root in patches of higher resource availability could enable clonal plants to benefit from absorption of soil resources in a better way compared to other plants (Yan *et al.*, 2013). This “foraging” mechanism could facilitate the plants to gain access to resources in rich patches in heterogeneous environments (de Kroon and Hutchings, 1995; de Kroon *et al.*, 2009) and optimise their exploitation of soil resources (de Kroon and Hutchings, 1995; Dong *et al.*, 1997; Whitlock *et al.*, 2010; Eilts *et al.*, 2011; Gough *et al.*, 2012; Oborny *et al.*, 2012; Martina and Ende, 2013). Alternatively, ramets can be produced by stolons at random, but the maternal investment could secure establishment even in poor quality patches.

In early stages of the growth of new ramets, there is transfer of resources from mother to daughter ramets through stolons (Walfer and Straile, 2012). It is common that more resources are transferred from rich mother ramets to daughter ramets in poor patches (Marba *et al.*, 2002; Saitoh *et al.*, 2006; Matlaga and Sternberg, 2009; Xu *et al.*, 2010; D’Hertefeldt *et al.*, 2011; He *et al.*, 2011). Alternatively, the transfer of resources might be from daughter ramets to mother ramets (Stuefer *et al.*, 1994; Peltzer, 2002), where the

mother ramets establishes more ramets in rich patches with a view to increasing e absorption (Martina and Von Ende, 2013).

This means that the mother ramets could “select” where the new daughter ramet establish, or they might be randomly deployed (de Kroon and Knops, 1990; de Kroon and Hutchings, 1995). The clonal integration mechanism varies depending on the plant species. For example, nutrients can move in two directions between mother and daughter ramets in some clonal plants, while in some species the nutrients only move from mother to daughter (Wolfer and Straile, 2012).

*Ochthochloa compressa* is a plant of local pastoral value. Recently, this plant disappeared from the surrounding area in the study region. Indeed, while the study area is protected from grazing by the government the area surrounding it is exposed to human activities such as grazing and urbanization stress. Field observations indicate that *O compressa* has the ability to spread by clonal mechanism. The high grazing value of *O compressa* and its ability to spread under harsh environmental conditions make it a very desirable plants species. As far as I know, *O. compressa* was not subject to research in the study area. Therefore, because of its high pastoral value and its ability to spread under the local harsh environmental conditions, the researcher was encouraged to study of the clonal integration mechanism in *O compressa* and its effect on it.

As suggested by previous studies, further research is required in order to get a better insight into both the clonal integration mechanism and the patterns of new ramet establishment mechanism. To understand the mechanism of spread of stolons and the establishment of new ramets in *O compressa*, I analysed the concentration of nitrogen, phosphorus and potassium in both mother and daughter ramets and the soil where they were located. The purpose of this is to inform the role of nutrient abundance in soil in the

establishment of new ramets and the spread of stolons. In this study, I aim to investigate whether mother ramets randomly establish new ramets or are the sites “chosen” by the mother ramets to ensure the best patches are occupied.

## Materials and methods

### *Study area*

The study field site was Al-Thumamah an area protected by the Saudi Arabian government. The Al-Thumamah area (N 25°11'8", E 46°38'2") is about 70 km from Riyadh, at an altitude is approximately 567 m, and its extension is approximately 170 km<sup>2</sup> (Fig. 1). The Al-Thumamah area contains mostly sandy soils, although rocky and gravelly soils can also be found. The vegetation is formed by scattered shrubs with perennial and annual herbaceous plants growing in between them.

The rainfall is almost 100 mm annually occurring almost exclusively between October and May (Fig. 2a). It has hence a very dry and hot climate with an average temperature in winter of 12 C°; while in summer temperatures reach 38 C° (Al Musallam, 2007) (Fig. 2b).

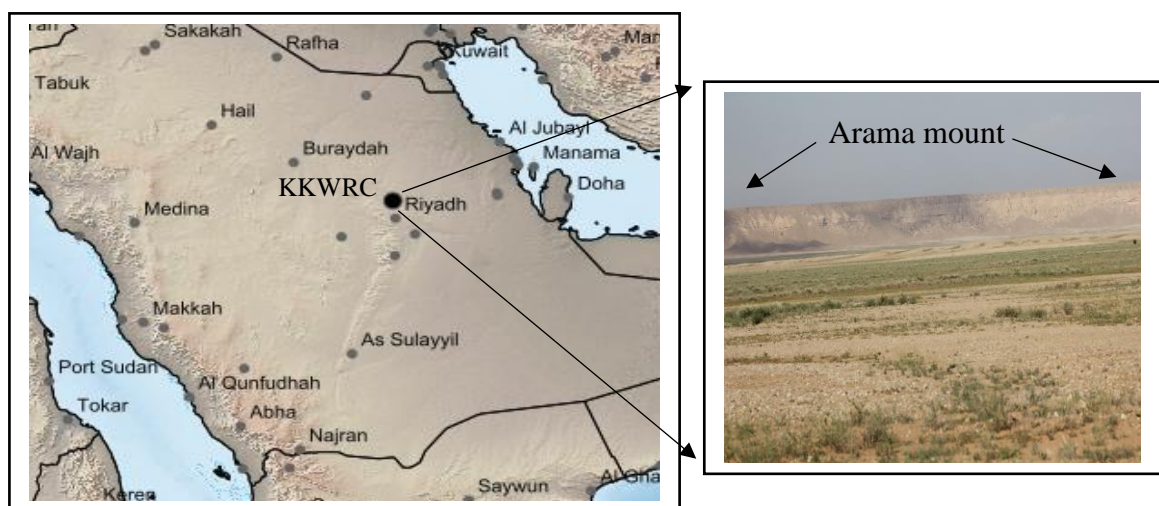
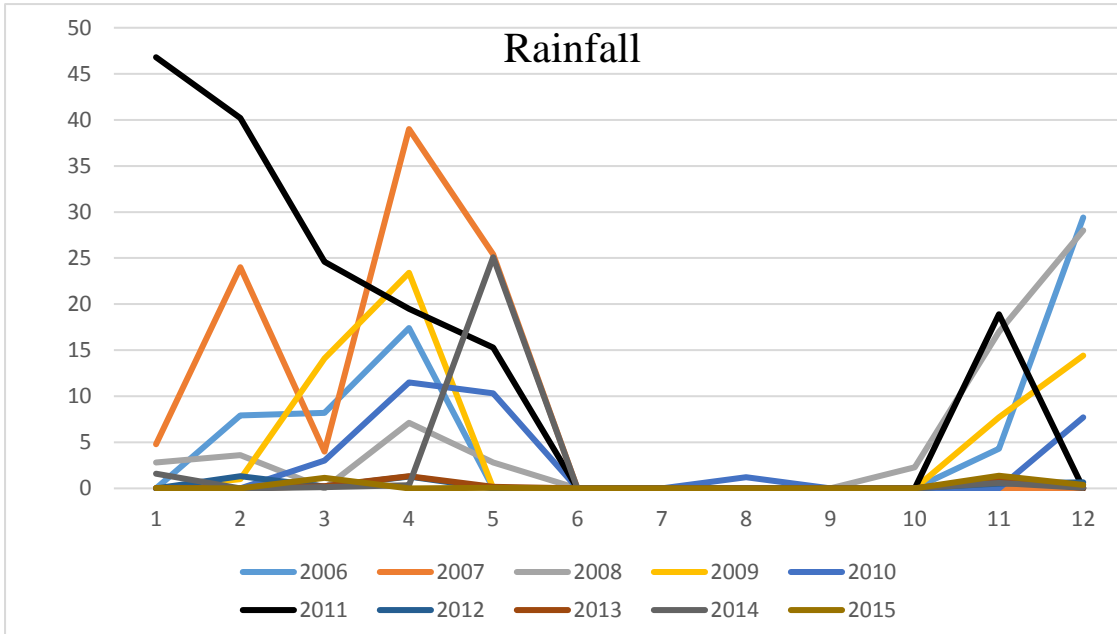
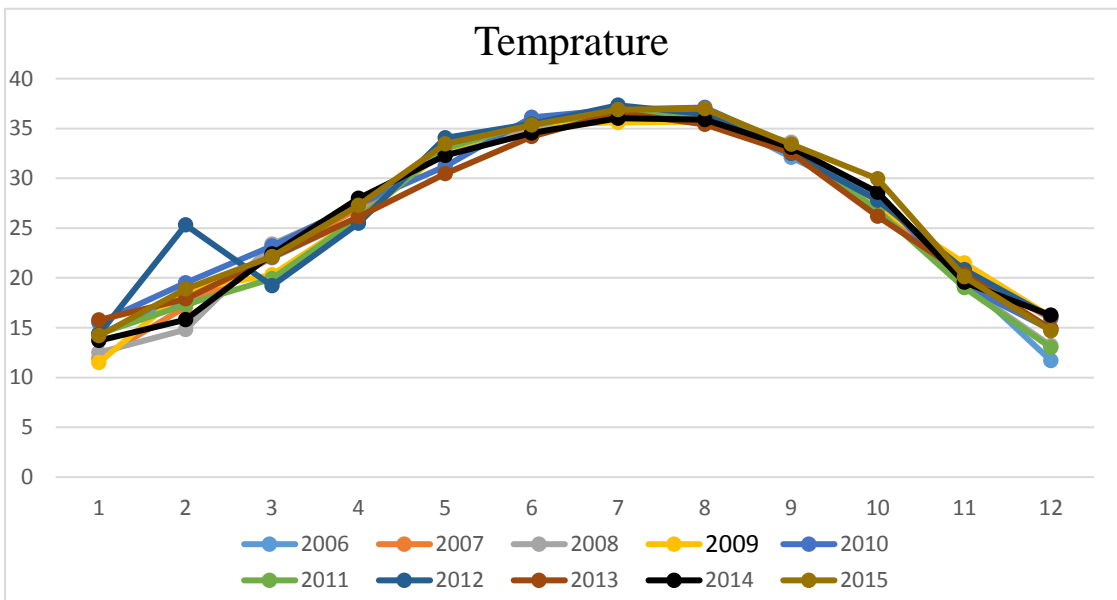


Figure. 1. The left picture for the King Khalid Wildlife Research Centre (KKWRC) area at Saudi Arabia map, and in right picture for study sites.



(Figure. 2a. The rate monthly rainfall over ten years from 2006 to 2015.)



(Figure. 2b. The monthly temperature over ten years from 2006 to 2015.)



### *Plant description*

*Ochthochloa compressa* is a perennial grass native to the Arabian Peninsula. The decumbent stolons can extend up to 150 cm, are 2 - 4 mm in diameter, sustain 1- 6 ramets each (personal observation) and it growth up to 70 cm tall (Collenette, 1999). The leaf-blades are conduplicate, 3 -5 cm long, 3 mm wide and glaucous. The lower leaves are longer than the higher leaves. It has two mechanism of reproduction, sexual by producing seeds in spikes and asexual by producing stolons that produce new ramets. Inflorescences contains 3 - 5 digitate racemes, 2 – 4 cm long, which are deciduous at maturity (Fig. 3). It produces seeds in February and March. *O compressa* is widespread in central, north and western Saudi Arabia (Collenette, 1999; Mandavilla, 1990), and extends into western Asia and Asia-tropical: India and Africa: North West tropical.



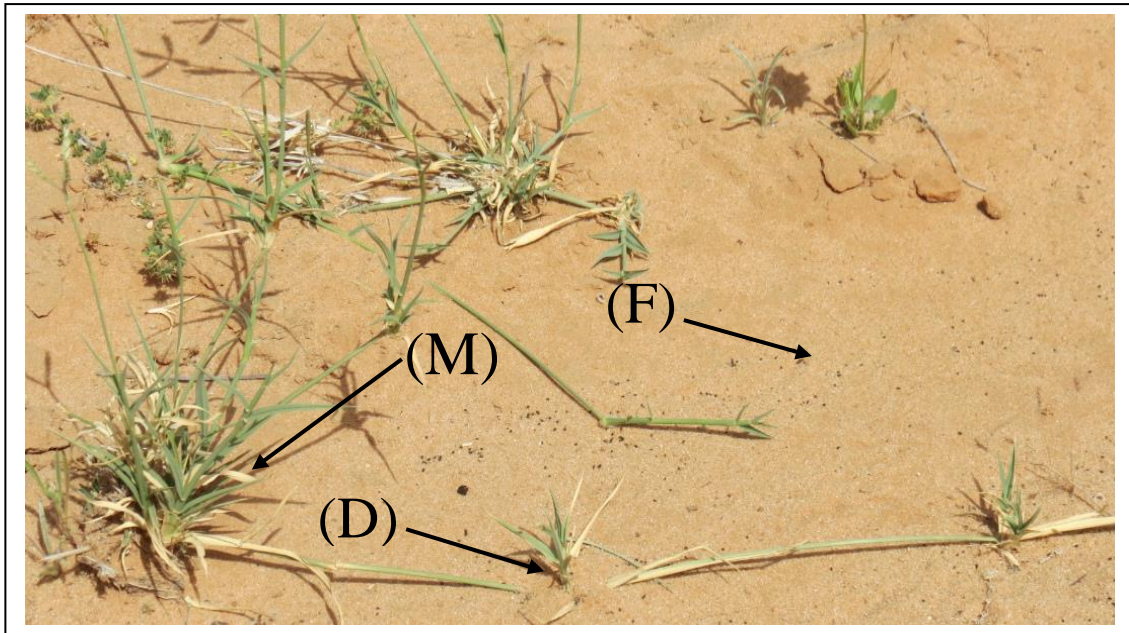
Figure. 3. The shape of spikes for *Ochthochloa compressa* (*Ochthochloa compressa* - Flora of Qatar 2016).

### *Data Collection*

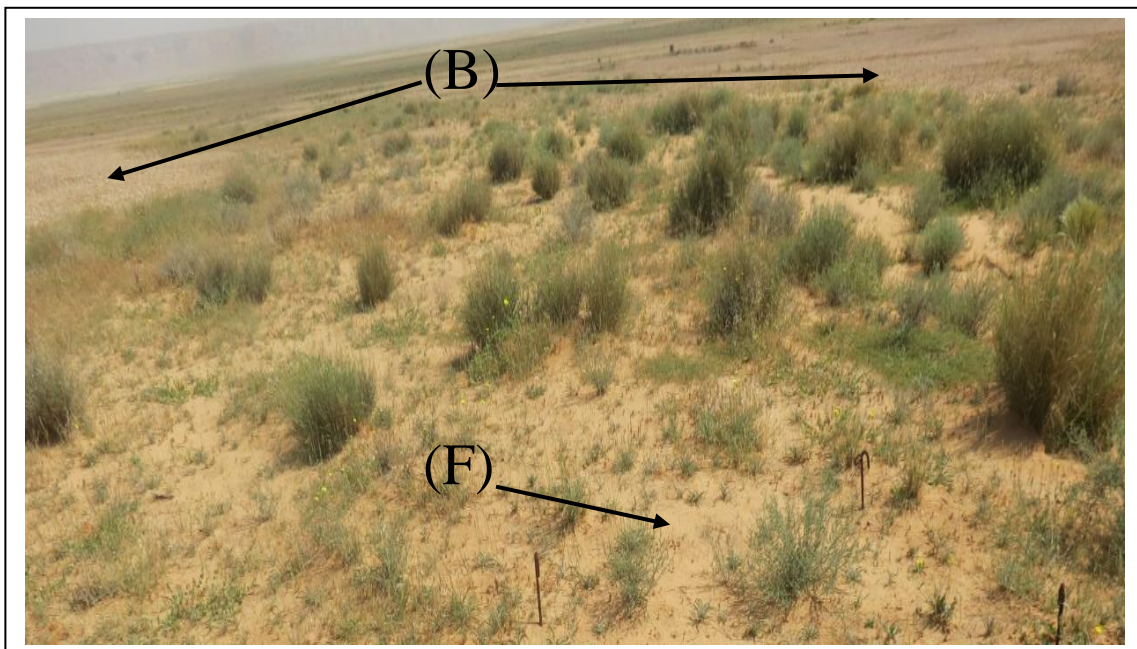
The study sampled six microsites representing different areas where the different parts of the plant were present or not, with nine replicates for each site, which are as follows:

1. Mother ramets (M) (Fig. 4a).
2. Daughter ramets (D) (Fig. 4a).

3. A node that has not produced root yet for this year (X1).
4. Unoccupied area surrounding the mother ramets (F) at 50 cm distance (Fig. 4a, b).
5. The bare area surrounding the study patch (B) (Fig. 4b).



(Figure. 4a. The sites for mother ramets, daughter rames and unoccupied sites).



(Figure. 4b. The sites for bare areas and unoccupied sites)

I collected our samples randomly from four sites (M, D, X1 and F) in first patch and fifth site (B) in bare patch in February 2014. I had been chosen 9 mother and daughter ramets for plant samples. As well as, I collected our samples from five sites, which are: mother ramets (M), daughter ramets (D), nodes that did not produce roots this year (X1), unoccupied area surrounded mother ramets (F), and bare area surrounding the study patch (B).

For soil sampling, I collected blocks  $10 \times 10 \text{ cm}^2$  and 5 cm deep without any treatment. I also collected leaf tissues of the corresponding plant part, in those microsites where plants were present. Thereafter, I dried the plant leaves in an electric oven at  $60 \text{ C}^\circ$  for 72 hours. Subsequently, I analysed the concentration of nitrogen, phosphorous, and potassium in the soil and in plants after grinding them. The laboratory at Ministry of Environment Water & Agriculture in Saudi Arabia analysed the soil samples using two methods. First, the Kjeldahl method was used to analyse the total nitrogen (Bremner and Mulvaney, 1982), second, the spectrophotometer method was used to analyse the total phosphorous and potassium (Olsem and Sommers, 1982).

### *Statistical analysis*

Our data showed equal variance. I therefore analysed the data using one-way ANOVA to determine if there was any difference in concentrations of the nutrients (nitrogen, phosphorous, and potassium) among all the soil samples (M, D, X1, F, and B). Correspondingly, I analyse the same concentrations for mother and daughter ramets. When ANOVA detected significant differences, I analysed the data in an unpaired t-test. I used that test to determine whether the daughter ramets are established randomly or whether they are determined by environmental factors.

## Result

The results for the total nitrogen concentration in the soil samples was found to be significantly different ( $P$  value  $< 0.0001$ ,  $F= 28.23$ ). The higher concentration of nitrogen were found in the F soil samples, while the B soil samples, recorded the lowest concentration of nitrogen. For the D and X1 soil samples, no significant difference was recorded. Also, soil samples F and M recorded the highest concentration of nitrogen, and no significant difference was found between both of them (Fig. 5).

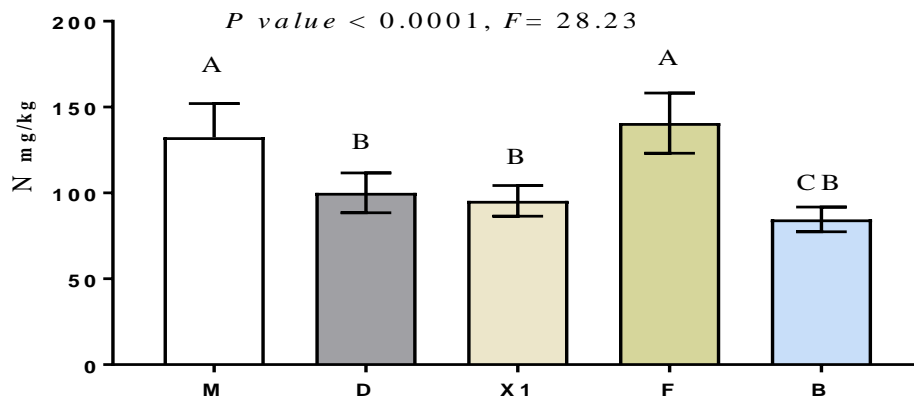


Figure.5. Concentration of nitrogen in soil samples for soil samples of mother ramets (M), daughter ramets which has root (D), daughter ramets which has not root (X1), unoccupied area surrounded mother ramets (F) and bare area surrounded patch study (B).

For total phosphorus in the soil samples, the result indicated a significant difference ( $P < 0.0001$ ,  $F= 23.64$ ). The highest concentration level was in the bare ground site (B), which recorded higher concentrations of phosphorus compared to the other site samples. The mother (M) and daughter (D) soil samples were recorded as having the lowest concentration level of phosphorus, with no significant difference between them. Also, there were no significant differences between B and all the other sites (Fig. 6).

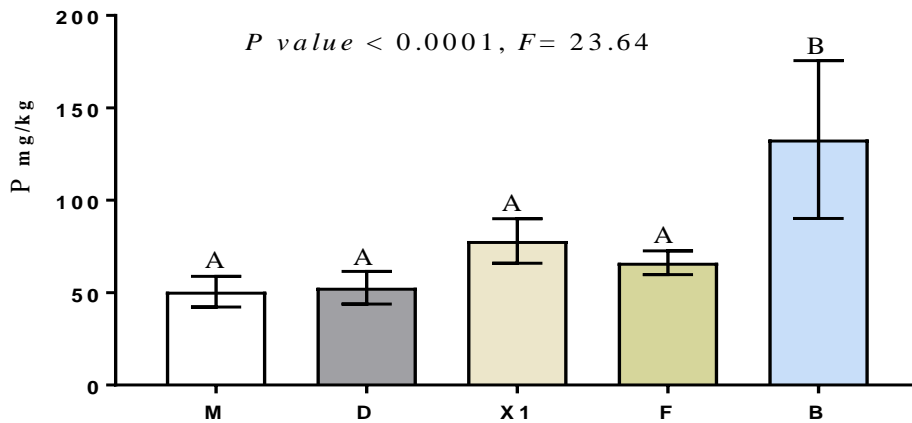


Figure.6. Concentration of phosphorus in soil samples for samples of mother ramets (M), daughter ramets which has root (D), daughter ramets which has not root (X1), unoccupied area surrounded mother ramets (F) and bare area surrounded patch study (B).

Significant differences were found for total soil potassium ( $P < 0.0001$ ,  $F = 16.94$ ) (Fig. 7). The lowest level of potassium was found in the X1 soil sample. The soil of F was significantly different, showing a higher concentration of potassium than other soil samples, but it was not different from N. No differences were found between the M, D, and B soil microsites, unlike X1, which was significantly different from all the other soil sites.

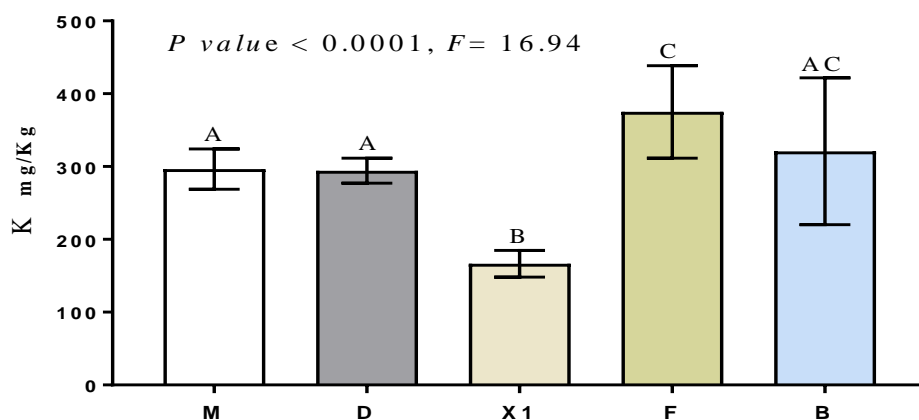
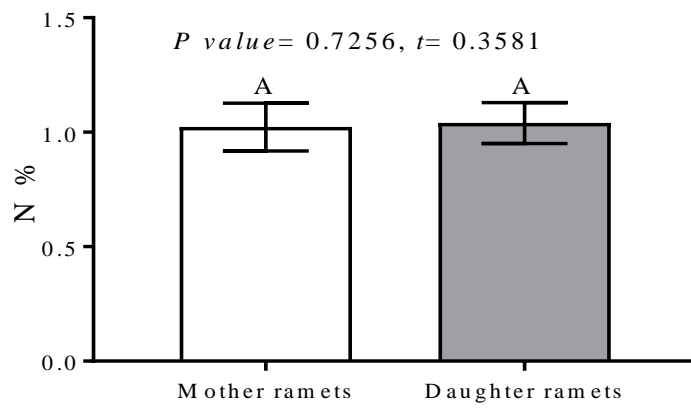
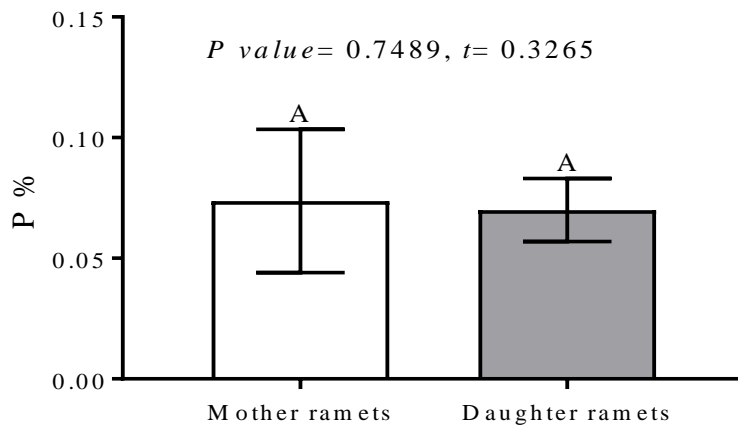


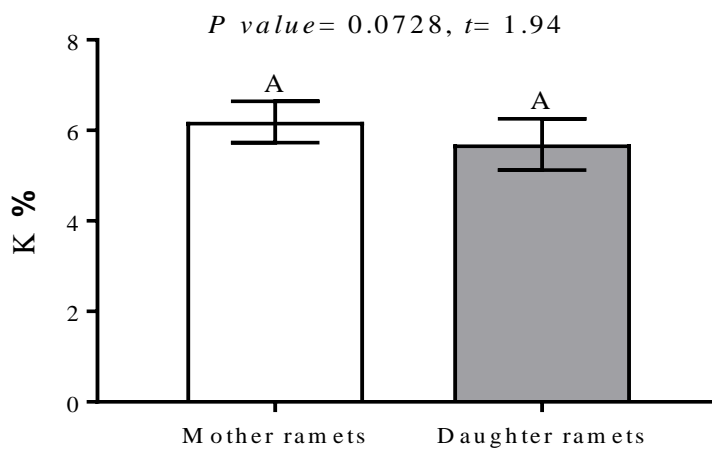
Figure.7. Concentration of potassium in soil samples for samples of mother ramets (M), daughter ramets which has root (D), daughter ramets which has not root (X1), unoccupied area surrounded mother ramets (F) and bare area surrounded patch study (B).



(a)



(b)



(c)

Figure. 8. Content of nitrogen (a), phosphorus (b) and potassium (c) in mother and daughter ramets.

None of the mother and daughter ramet samples showed any significant difference in nitrogen, phosphorus and potassium concentration (Fig. 8 a, b, and c) (nitrogen  $P = 0.7256$ ,  $t = 0.3581$ ; phosphorus  $P = 0.7489$ ,  $t = 0.3265$ ; and potassium  $P = 0.0728$ ,  $t = 1.94$ ).

## Discussion

There was a heterogeneous distribution of nutrients in the soil among the sites sampled. The nutrient concentration in areas where the plant does not grow was much higher, probably because of finer soils. These soils are also likely to be shallower and not conducive to the growth of *O. compressa*. The nitrogen concentration in the area where the plants grow showed a significant difference. The soil samples for mother ramets and unoccupied area (within 50 cm distance) recorded the highest concentration of nitrogen. Contrary to our expectation, the soil samples under daughter ramets recorded a lowest concentration. The mother ramet's samples recorded a higher concentration of nitrogen than the daughter ramet's samples, which might be because of the mother ramets' sufficiency of roots size at the age of more than a year. Mother plants are actually likely to be much older and might have also accumulated fine particles and organic matter enriching the patch. The spread of stolons and the establishment of the daughter ramets were done randomly, despite the unoccupied area, which was recorded higher concentrations than daughter ramets soil samples of nitrogen concentration, which indicated that nitrogen concentration in the soil does not affect the spread of the stolons. This clearly indicates that, the spread mechanism was a random process (de Kroons and Hutchings, 1995).

The patch where the mother and ramets are located was on sandy soil, distant from the bare patch which has a cohesive soil that contains gravel and fine materials deposited by water flows. Phosphorus analysis result showed that significant differences between the

patch where the ramets and the bare patch. Also, the bare patch recorded a higher concentration of phosphorus compared to the other patch. The cause of that might be the nature of the soil. Also, the difficulty of movement of water to soil depth, could lead to increase the concentration of phosphorus in soil. While the other patch has sandy soils which allows water to reach the soil depth and can contribute to leach of the soil, and lead to phosphorus to move in soil and enable plants to absorb it.

Potassium analysis results showed significant differences. Potassium concentration varied among soil samples. However, X1 soil samples showed the lowest concentration of potassium compared with other samples. Unfortunately, I could not find on a specific reason to low the concentration of potassium in X1 samples. Further research contemplating this pattern is warranted.

No significant differences were found between nitrogen, phosphorus, and potassium concentrations in the mother and daughter ramets in spite of the differences in the soil they were growing in. This indicates the ability of mother ramets to support the other ramets, and suggests that clonal integration averages the soil differences where the different ramets are growing. More information about the behaviour of plant integration (e.g what determined the distribution of nutrient between ramets) needs further study (Bartels and Chen, 2010; He *et al.*, 2011; Mommer *et al.*, 2011; van der *et al.*, 2011).

The current results show that the *O. compressa* spreads randomly through stolons and establish the new ramets randomly or even perhaps in patches with lower levels of nutrients. The mother plant, however, seems to provide the ramets with abundant nutrients, since I found no difference in nutrient concentration between mother growing in better patches and the daughters growing in poorer soils. This is consistent with what de Kroon and Knops (1990), and de Kroons and Hutchings (1995) found that the mother



ramets produce stolons and randomly establish daughter ramets. This however contrasts with Alpert (1999), Alpert *et al.* (2003) and Nilsson and D'Hertefeldt (2008) who found that a mother ramet “chooses” the places of daughter ramets and the spread of the stolons based on the abundance of nutrients and resources.

These results contribute to understand the behaviour of *O. compressa* as one of the clonal plants. In conclusion, I recommend that further research and study of the phenomenon of integration to explain the distribution mechanism of clonal plants, spread it and the composition of the plant environment (Maurer and Zedler, 2002; Martina and Von Ende, 2013).

## References

- Al Musallam, MS** 2007, 'Effect of Protection on Vegetation and Soil Seed Bank in Thumama Area' MA thesis, King Saud University, Riyadh.
- Alpert, P & Mooney, H** 1986, 'Resource sharing among ramets in the clonal herb, *Fragaria chiloensis*', *Oecologia*, vol. 70, no. 2, pp. 227-233.
- ALPERT, P** 1996. 'Nutrient sharing in natural clonal fragments of *Fragaria chiloensis*'. *Journal of Ecology* 84: 395–406.
- Alpert, P** 1999, 'Clonal integration in *Fragaria chiloensis* differs between populations: ramets from grassland are selfish', *Oecologia*, vol. 120, no. 1, pp. 69-76.
- Alpert, P, Holzapfel, C & Slominski, C** 2003, 'Differences in performance between genotypes of *Fragaria chiloensis* with different degrees of resource sharing', *Journal of Ecology*, vol. 91, no. 1, pp. 27-35.
- Bartels S &, Chen, HY** 2010. Is understory plant species diversity driven by resource quantity or resource heterogeneity? *Ecology*, vol. 91, no. 7, pp. 1931-1938.
- Bremner, J.M &., Mulvaney, C.S** 1982. Methods of soil analysis, part 2 chemical and Micro biological properties, 595-624.

- Brewer, JS & Bertness, MD** 1996, 'Disturbance and intraspecific variation in the clonal morphology of salt marsh perennials', *Oikos*, pp. 107-116.
- Chen JS, Lei NF, & Dong M** 2010. 'Clonal integration improves the tolerance of *Carex praeclara* to sand burial by compensatory response'. *Acta Oecologica*, vol. 36, no. 1, pp. 23-28.
- Clark, JS, Bell, D, Chu, C, Courbaud, B, Dietze, M, Hersh, M, HilleRisLambers, J, Ibáñez, I, LaDeau, S & McMahon, S** 2010, 'High-dimensional coexistence based on individual variation: a synthesis of evidence', *Ecological Monographs*, vol. 80, no. 4, pp. 569-608.
- Collenette, S** 1999, 'Wildflowers of Saudi Arabia', National Commission for Wildlife Conservation and Development (NCWCD), Riyadh.
- D'Hertefeldt, T, Falkengren-Grerup, U & Jónsdóttir, I** 2011. 'Responses to mineral nutrient availability and heterogeneity in physiologically integrated sedges from contrasting habitats', *Plant Biology*, vol. 13, no. 3, pp. 483-492.
- de Kroon, H & Knops, J** 1990, 'Habitat exploration through morphological plasticity in two chalk grassland perennials', *Oikos*, pp. 39-49.
- de Kroon, H, Visser, EJ, Huber, H, Mommer, L & Hutchings, MJ** 2009, 'A modular concept of plant foraging behaviour: the interplay between local responses and systemic control', *Plant, Cell & Environment*, vol. 32, no. 6, pp. 704-712.
- de Kroon, H & Hutchings, MJ** 1995, 'Morphological plasticity in clonal plants: the foraging concept reconsidered', *Journal of Ecology*, pp. 143-152.
- Dong M, During H, & Werger M** 1997. 'Clonal plasticity in response to nutrient availability in the pseudoannual herb, *Trientalis europaea* L'. *Plant Ecology*, vol. 131, no. 2, pp. 233-239.

- Eilts JA, Mittelbach GG, Reynolds HL ,& Gross KL** 2011. 'Resource heterogeneity, soil fertility, and species diversity: effects of clonal species on plant communities'. *The American Naturalist*, vol. 177, no. 5, pp. 574-588.
- Evans, J. P** 1988. 'Nitrogen translocation in a clonal dune perennial, *Hydrocotyle bonariensis*'. *Oecologia*, vol. 77, no. 1, pp. 64-68.
- Gough L, Gross KL, Cleland EE, Clark CM, Collins SL, Fargione JE, Pennings SC & Suding KN** 2012. 'Incorporating clonal growth form clarifies the role of plant height in response to nitrogen addition'. *Oecologia*, vol. 169, no. 4, pp. 1053-1062.
- He W-M, Alpert P, Yu F-H, Zhang L-L ,& Dong M** 2011. 'Reciprocal and coincident patchiness of multiple resources differentially affect benefits of clonal integration in two perennial plants'. *Journal of Ecology*, vol. 99, no. 5, pp. 1202-1210.
- Luo, W & Zhao, W** 2015, 'Effects of wind erosion and sand burial on growth and reproduction of a clonal shrub', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 217, pp. 164-169.
- Mandavilla, J. P** 1990, 'flora of Eastern Saudi Arabia', Kegan Paul International Limited, London.
- Marba N, Hemminga MA, Mateo MA, Duarte CM, Mass YEM, Terrados J & Gacia, E** 2002. 'Carbon and nitrogen translocation between seagrass ramets'. *Marine Ecology Progress Series*, vol. 226, pp. 287-300.
- Martina, J & Von Ende, C** 2013, 'Increased spatial dominance in high nitrogen, saturated soil due to clonal architecture plasticity of the invasive wetland plant, *Phalaris arundinacea*', *Plant Ecology*, vol. 214, no. 12, pp. 1443-1453.
- Matlaga DP & Sternberg LDL** 2009. 'Ephemeral clonal integration in *Calathea marantifolia* (Marantaceae): evidence of diminished integration over time'. *American journal of botany*, vol. 96, no. 2, pp. 431-438.

- Maurer, DA & Zedler, JB** 2002, 'Differential invasion of a wetland grass explained by tests of nutrients and light availability on establishment and clonal growth', *Oecologia*, vol. 131, no. 2, pp. 279-288.
- Mommer L, Wisser, Wisser EJW, van Ruijven J, de Caluwe H, Pierik R & de Kroon H** 2011. 'Contrasting root behaviour in two grass species: a test of functionality in dynamic heterogeneous condition'. *Plant and soil*, vol. 344, no. 1-2, p. 347.
- Nilsson, J & D'Hertefeldt, T** 2008, 'Origin matters for level of resource sharing in the clonal herb *Aegopodium podagraria*', *Evolutionary Ecology*, vol. 22, no. 3, pp. 437-448.
- Oborny B, Mony C ,& Herben T** 2012. 'From virtual plants to real communities: a review of modelling clonal growth'. *Ecological Modelling*, vol. 234, pp. 20-30.
- Ochthochloa compressa - Flora of Qatar*** 2016, Flora of Qatar, viewed 6 April 2017, <[http://www.floraofqatar.com/ochthochloa\\_compressa.htm](http://www.floraofqatar.com/ochthochloa_compressa.htm)>.
- Olsem, S.R., Sommers, L.E** 1982. Phosphorus. In: A.L. Page, R.H. Miller (Eds). *Methods of Soil Analysis. Part 2.* 2nd ed. Agronomy Monograph 9, ASA and SSSA, Madison, WI, pp. 403-430.
- Peltzer, DA** 2002, 'Does clonal integration improve competitive ability? A test using aspen (*Populus tremuloides* [Salicaceae]) invasion into prairie', *American journal of botany*, vol. 89, no. 3, pp. 494-499.
- Pennings, SC & Callaway, RMENNINGS, S. C., AND R. M. CALLAWAY** 2000. 'The advantages of clonal integration under different ecological conditions: a community-wide test'. *Ecology*, vol. 81, no. 3, pp. 709-716.

- Saitoh T, Seiwa K, & Nishiwaki A** 2006. 'Effects of resource heterogeneity on nitrogen translocation within clonal fragments of *Sasa palmata*: an isotopic ( $^{15}\text{N}$ ) assessment'. *Annals of Botany*, vol. 98, no. 3, pp. 657-663.
- Salzman, A. G., & M. A. Parker, M. A** 1985. 'Neighbors ameliorate local salinity stress for a rhizomatous plant in a heterogeneous environment'. *Oecologia*, vol. 65, no. 2, pp. 273-277.
- Slade, A.J. & Hutchings, M.J** 1987. 'The effects of nutrient availability on foraging in the clonal herb *Glechoma hederacea*'. *The Journal of Ecology*, pp. 95-112.
- Stuefer JF, During HJ & De kroon H** 1994. 'High benefits of clonal integration in two stoloniferous species, in response to heterogeneous light environments'. *Journal of Ecology*, pp. 511-518.
- Stuefer J. F &, Hutchings, M.JM. J** 1994. 'Environmental heterogeneity and clonal growth: a study of the capacity for reciprocal translocation in *Glechoma hederacea* L'. *Oecologia*, vol. 100, no. 3, pp. 302-308.
- Sutherland, W.J. & Stillman, R.A** 1988. 'The foraging tactics of plants'. *Oikos*, 52, 239-244.
- van der Waal, C, de Kroon, H, Heitkönig, I, Skidmore, AK, van Langevelde, F, de Boer, WF, Slotow, R, Grant, RC, Peel, MP & Kohi, EM** 2011, 'Scale of nutrient patchiness mediates resource partitioning between trees and grasses in a semi-arid savanna', *Journal of Ecology*, vol. 99, no. 5, pp. 1124-1133.
- Whitlock R, Grime JP ,& Burke T** 2010. 'Genetic variation in plant morphology contributes to the species-level structure of grassland communities'. *Ecology*, vol. 91, no. 5, pp. 1344-1354.

- Wijesinghe, D. A & ., Hutchings, M.J M.** 1997. 'The effects of spatial scale on the growth of a clonal plant: an experimental study with *Glechoma hederacea*'. *Journal of Ecology*, pp. 17–28.
- Wolfer, SR & Straile, D** 2012. 'To share or not to share: clonal integration in a submerged macrophyte in response to light stress', *Hydrobiologia*, vol. 684, no. 1, pp. 261-269.
- Xu CY, Schooler SS & Van Klinken RD** 2010. 'Effects of clonal integration and light availability on the growth and physiology of two invasive herbs'. *Journal of Ecology*, vol. 98, no. 4, pp. 833-844.
- Yan, X, Wang, H, Wang, Q & Rudstam, LG** 2013. 'Risk spreading, habitat selection and division of biomass in a submerged clonal plant: Responses to heterogeneous copper pollution', *Environmental pollution*, vol. 174, pp. 114-120.
- Yu, F.H., Wang, N., He, W.M., Chu, Y., & Dong, M** 2008. 'Adaptation of rhizome connections in drylands: increasing tolerance of clones to wind erosion'. *Annals of Botany*, vol. 102, no. 4, pp. 571-577.

## Chapter 3

# The effect of abundance of nutrients in soil on the spread of mother ramet's stolons and on the potential establishment of a new ramets.

### Introduction

Clonal integration allows the sharing of soil resources, such as water and nutrients, and carbohydrates between ramets that are connected to each other (de Kroon and Knops, 1990; Cain, 1994; de Kroon and Hutchings, 1995; Evans and Cain, 1995; Brewer and Bertness, 1996; Stuefer *et al.*, 1996; Hutchings and Wijesinghe, 1997; Charpentier *et al.*, 1998; Stoll *et al.*, 1998; Pennings and Callaway, 2000; Yu *et al.*, 2004). Also, clonal integration can help exploit soil resources more efficiently by exploiting patches where different resources are concentrated and hence improve the competitiveness of the clonal plant (Peltzer, 2002). Integration also helps tolerate the stress caused by the surrounding environmental conditions (Slade and Hutchings, 1987; Hartnett and Bazzaz, 1985), such as resistance to wind erosion as documented for *Calligonum araboescens* (Luo and Zhao, 2015).

The level of benefit from clonal integration varies among plant species (He *et al.*, 2011), since the variation in distribution of nutrients plays a role in the spread of plants and their vegetation structure in patches (He *et al.*, 2011). The heterogeneity of the habitat can determine how clonal integration improves the ability of a plant to distribute and share resources among connected ramets, which suggests a plant's ability to choose suitable habitats that provides enough sustenance to survive (Alpert, 1999; Nilsson and D'Hertefeldt, 2008; He *et al.*, 2011).

Mother ramets typically supply nutrients to daughter ramets (Caraco and Kelly, 1991; Alpert, 1999) through a stolon or rhizome. The connection between mother and daughter ramets may continue for a year or even years until the ramets produce roots that allow them to sustain themselves (Alpert and Mooney, 1986; He *et al.*, 2011). Connection may be essential for the success of the daughter ramet even after the time when potentially it could become independent.

When the stolon between mother and daughter ramets were disconnected after daughter ramets were already well established, and hence it could had been expected that this would not effect on the chances of survival of the daughter, it still caused the weakening of growth (Peltzer, 2002). Even in harsher patches daughter ramets failed to grow because they were not capable of obtaining enough water and nutrients from the soil (He *et al.*, 2011). In addition, the nutrients were distributed randomly and homogeneously in the soil for individual plants, thereby the ability to establish roots depends on the availability of different nutrient in the soil (Bartels and Chen, 2010; He *et al.*, 2011, 2012; Mommer *et al.*, 2011).

The distribution of nutrients in the soil might affect the distribution of plants and their expansion. Unfortunately, there is not enough information yet about the role and benefits of clonal integration for the clonal plant under different environmental conditions (Pennings and Callaway, 2000). Actually, studies in relatively favourable environments, or environments where light is the main limiting resource prevails in the literature, while arid lands are relatively underrepresented. Hence, the study area (central Arabia Peninsula), suffering from a very dry environment, unregulated grazing, poor soil and extremely high temperature in summer and low temperature in winter, provides an excellent environment to test the benefits of clonal integration. I have also identified one species in the area (*Ochthochloa compressa*) as an excellent study species because of its



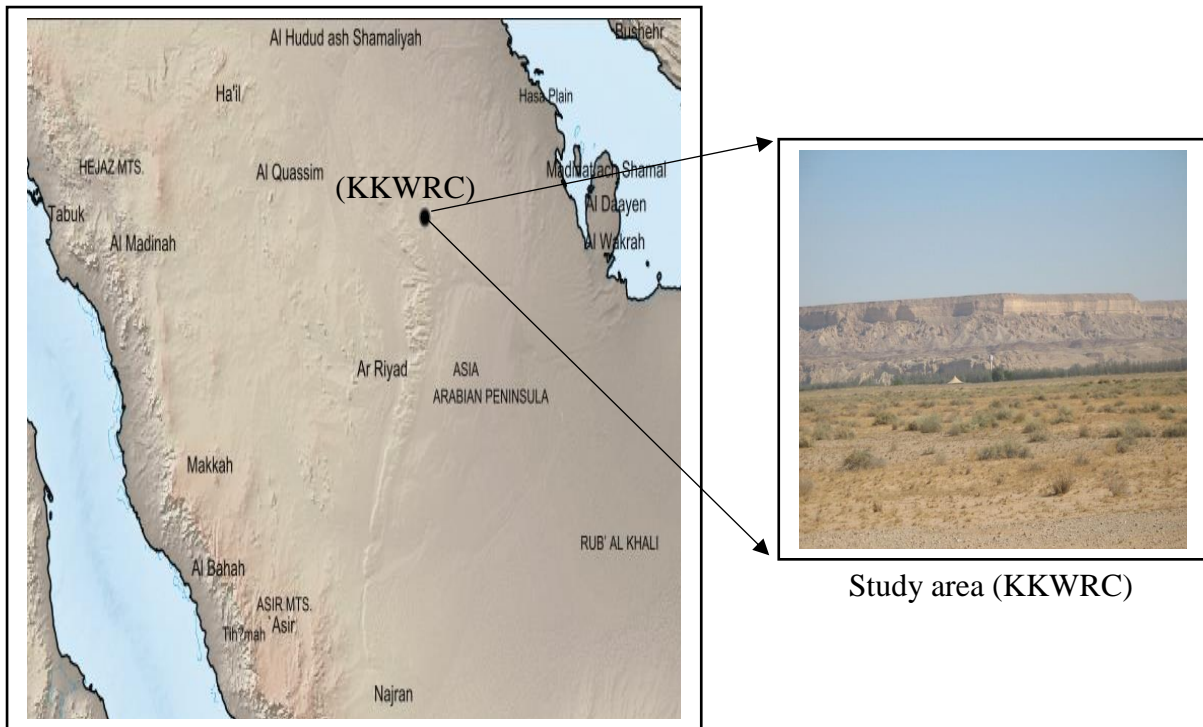
tolerance to the extreme environmental conditions of the study area as well as its ability to resist grazing, being highly palatable to grazing animals such as camels. How *Ochthochloa compressa* uses clonal integration to persist in an environment under grazing and the harsh local environmental conditions need further study.

This study aimed to investigate the effect of abundance of nutrients in soil on the relationship between mother and daughter ramets through analysis the concentration of content of N, P and K in both of mother ramets (M) and daughter ramets (D). Also, the impact of that on the biomass, efficiency of photosynthetic and the growth rate in both of them was investigated

## Materials and methods

### *Study area*

The study area was Al-Thumamah (N 25°11'05.43", E 46°41'48.09") located 70 km from Riyadh city (Fig. 1), at an altitude of approximately 570 m above sea level, and with an extension of approximately 170 km<sup>2</sup>. Al-Thumamah contains mostly sandy soil with some scattered areas with exposed rocks and gravely soil (Al Musallam, 2007). The climate is dry with average rainfall around 100 mm annually and occurs almost between October and May (Fig. 2). Also, the climate is a dry climate and the average of temperature in winter is 7 C° and in summer temperatures reach 38 C° (Fig. 3).



(Figure. 1. The left picture for the King Khalid Wildlife Research Centre (KKWRC) area at Saudi Arabia map, and in right picture for study sites).

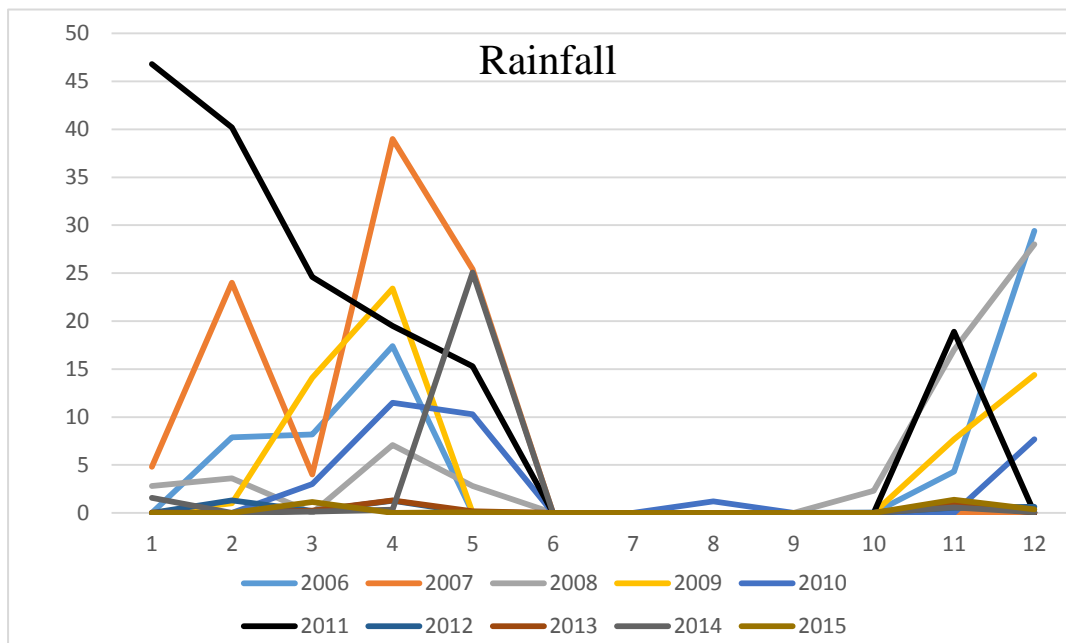
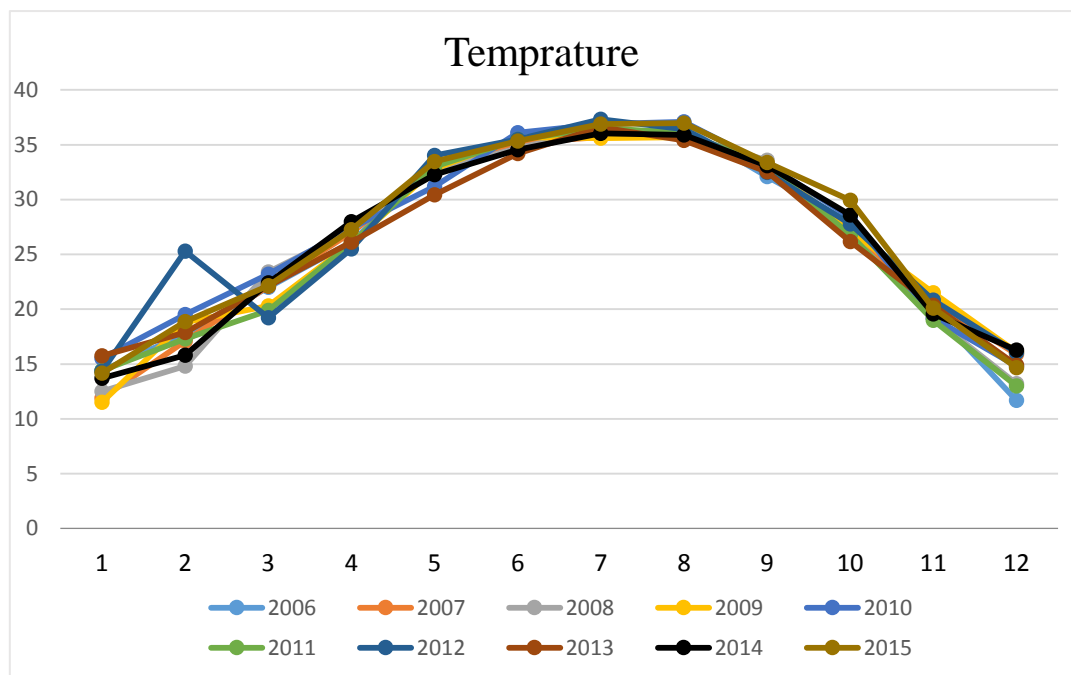


Figure. 2. The rainfall monthly rate between 2006 to 2015.



(Figure. 3. The temperature monthly rate among 2006 to 2015).

### *Plant description*

*Ochthochloa compressa* is a perennial grass. It growth up to 70 cm (Collenette, 1999), and the decumbent stolons can extend up to 150 cm, being 2 - 4 mm in diameter, connecting 1- 6 daughter ramets to each mother plant (personal observation). Leaf-blades are conduplicate, 3 -5 cm long, 3 mm wide and glaucous, lower leaves are longer than the upper leaves. It has two mechanism of reproduction: sexual way by producing seeds in spikes, and asexual by producing stolons from which ramets arise. The inflorescences contain 3 - 5 digitate racemes, 2 – 4 cm long. These are deciduous at maturity (Fig. 4). It produces seeds in February and March. *O compressa* is widespread in the central, north and the Arabian Peninsula (Mandavilla., 1990; Collenette, 1999), western Asia: India and North West Africa.



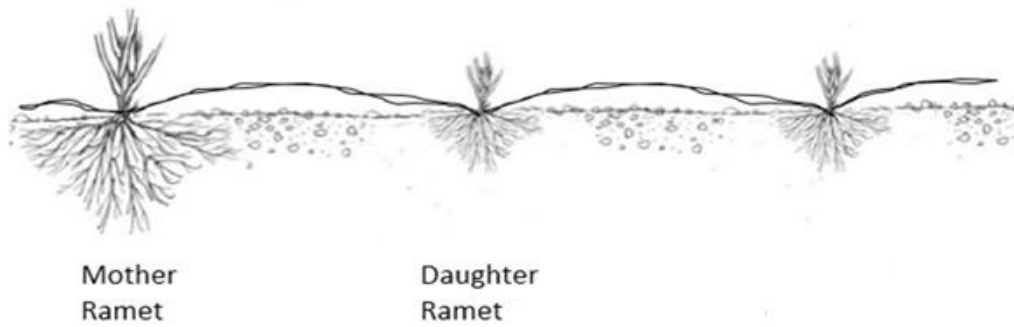
(Figure. 4. The left top picture for daughter ramets, the left bottom for *Ochthochloa compressa* spike, the right top picture for stolon length and the right bottom picture for mother ramets .)

### *Data collection*

I had four treatments with eight replicates, each consisting of a mother plant with at least 8 daughter plants attached to stolons. Plants were randomly selected in four different sites or patches. To randomly selected plants I added fertilizer to either Mother or Daughter ramets in four different treatments.

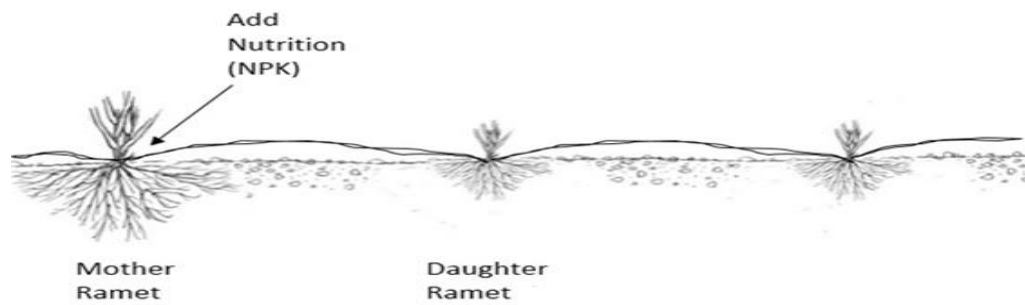
Our treatments were started in the first week in February 2014 and the plant were harvested on last week in March. The commercial fertilizer used contained about 20% N, 20% P, 20% K and 40% of other elements (such as calcium, sulphur etc.). I added 200 ml of fertilizer solution (200 gm/ 1L of distilled water) for each plant (Mother and Daughter ramets) weekly. The treatments were as follows:

1. Control without adding any fertilizer (Fig. 5).



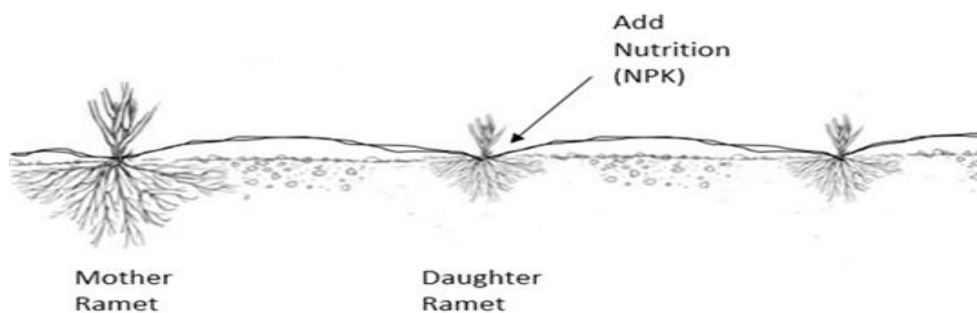
(Figure. 5. Control treatment.)

2. Adding fertilizer to the Mother ramet while connected to Daughter ramets (Fig. 6).



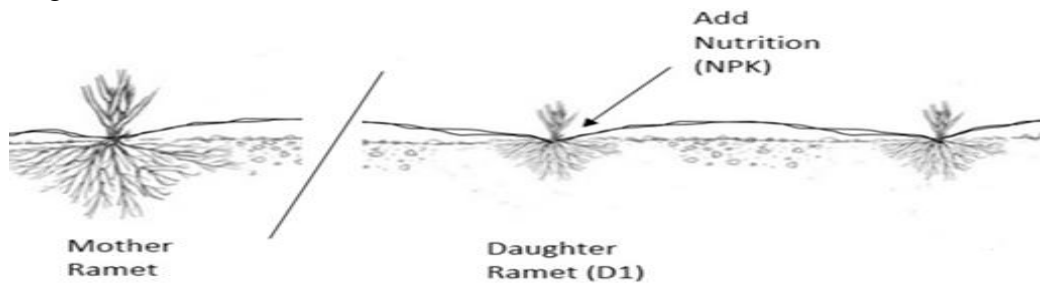
(Figure. 6. Add fertilizer to mother ramets while connection treatment.)

3. Adding a fertilizer to Daughter ramets while connected to the Mother ramet (Fig. 7).



(Figure. 7. Add fertilizer to daughter ramets while connection treatment.)

4. Adding a fertilizer to Daughter ramets while disconnected in Mother ramets (Fig. 8).



(Figure. 8. Add fertilizer to daughter ramets while disconnection treatment.)

I looked at the effect of nutrient addition on concentration of N, P, and K, in biomasses, efficiency of photosynthetic ( $F_v/F_m$ ) and the growth rate in both mother and daughter ramets. I used an OS30p device (Fig. 9) for measurement of  $F_v/F_m$  on young, fully extended leaves by placing the dark adaption leaf clips for 20 minutes, then measured using OS30p device (Fig. 10). Subsequently, I harvested all vegetative tissues for all mother and daughter ramets for biomass measurements. The material was then dried in oven at  $60\text{ }^\circ\text{C}$  for 48 hours, and weighted. Afterwards I grounded it to be sent to a laboratory at Ministry of Environment Water & Agriculture in Saudi Arabia for analyses of all samples.

The samples were analysed by Kjeldahl method to estimate the total N (Bremner and Mulvaney, 1982) and estimate the total P and K by Spectrophotometer methods (Olsem and Sommers, 1982).



Figure. 9. OS30p device (OS-30p+ / Worldwide Trade Thai n.d.).



Figure. 10. The dark adaption leaf

Also, I measured the growth rate by measuring a plant high at the beginning (H1) and at the end (H2) of experiments for mother and daughter ramets, and then calculated the rate of elongation.

$$\left( \frac{H2-H1}{H1} \right).$$

### Statistical analysis

Diagnosis of data, showed that the variance of all data were homogenous. One Way Anova was hence used to test for differences in Mother and Daughter ramets on all soil and plant samples for analysis total nitrogen (N), phosphorus (P) and potassium (K). Similar analyses were used to test the effect of fertilizer on biomasses, concentration of NPK and Photosynthesis performance. When ANOVA detected significant differences, I analysed the data in an unpaired t-test. I used that test to determine whether the daughter ramets are established randomly or whether they are determined by environmental factors.

## Results

The control treatments show that the contents of N, P and K were not significantly different between mother and daughter (Figs. 11a, b, c;  $P > 0.05$ ), consistent with the results from the previous chapter.

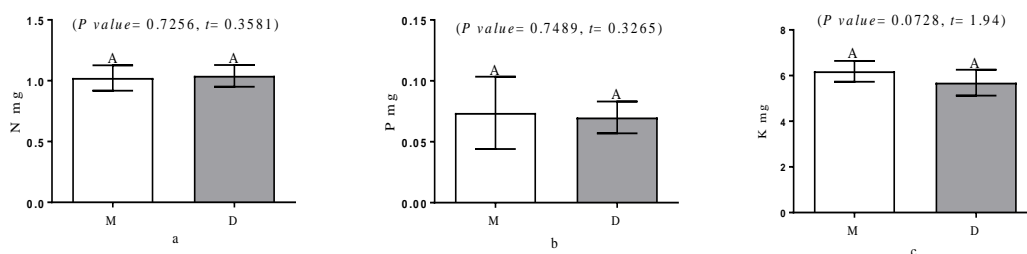


Figure. 11. Contents of N, P and K in mother (M) and daughter (D) ramets in control treatment (without adding a fertilizer), (a) Nitrogen, (b) Phosphorus, (c) Potassium of mother (M; white bar) & daughter (D; grey bar).

When nutrients were added to the mother plant while connected to the daughter, N was significantly different between M and D ( $P$  value = 0.0252,  $t$  = 2.469,  $df$  = 16), whereby the N content of D (1.04 +/- 0.09) was higher than that in M (1.02 +/- 0.11) (Fig. 12a). In addition, P was significantly different between M and D ( $t$  = 5.468,  $df$  = 16,  $P$  value <0.0001), whereby the P mean of D was (0.07 +/- 0.03) (Fig. 12b). However, K was not significantly different between M and D ( $P$  value= 0.3256,  $t$ = 1.014,  $df$ = 16) (Fig. 12c).

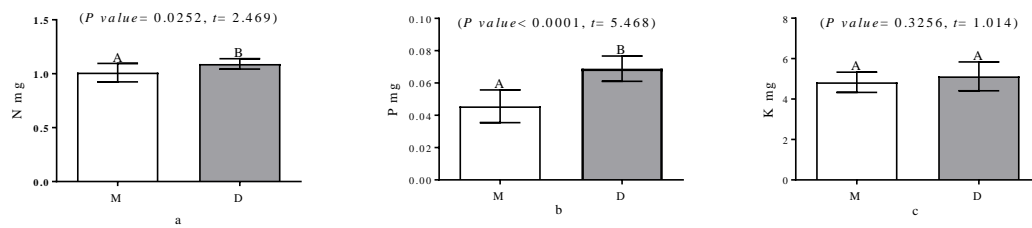


Figure. 12. Contents of N, P and K in mother and daughter ramets in adding fertilizer to mother ramets while connected daughter ramets treatment, (a) Nitrogen, (b) Phosphorus, (c) Potassium of mother (M; white bar) & daughter (D; grey bar).

When I added the nutrients to daughter ramets while connected to mother stolons, the results showed that N was significantly different between M and D ( $P$  value < 0.0001,  $t$  = 12.89,  $df$  = 16), whereby the N mean of D (1.12 +/- 0.01) was higher than that in M (0.94 +/- 0.04) (Fig. 13a). Nevertheless, the P and K concentrations were not statistically significantly different between M and D ( $P$  > 0.05) (Fig. 13b, c).

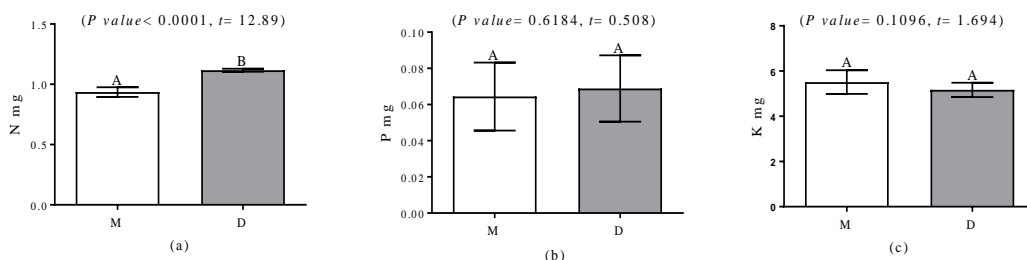


Figure. 13. Contents of N, P and K in mother and daughter ramets in adding a fertilizer to daughter ramets while connected mother ramet, (a) Nitrogen, (b) Phosphorus, (c) Potassium of mother (M; white bar) & daughter (D; grey bar).



As for biomass, the results showed no significant difference in main effects not in interaction amongst mother ramets ( $P$  value = 0.93,  $F$  = 0.07274) in three treatments (control, adding nutrients to mother ramets, and adding nutrients to daughter ramets) (Fig. 14 a). Also, there was no significant difference in daughter ramets ( $P$  value = 0.7148,  $F$  = 0.3047) in either of the three treatment (control, adding nutrients to mother ramets, and adding nutrient to daughter ramets) (Fig. 14 b).

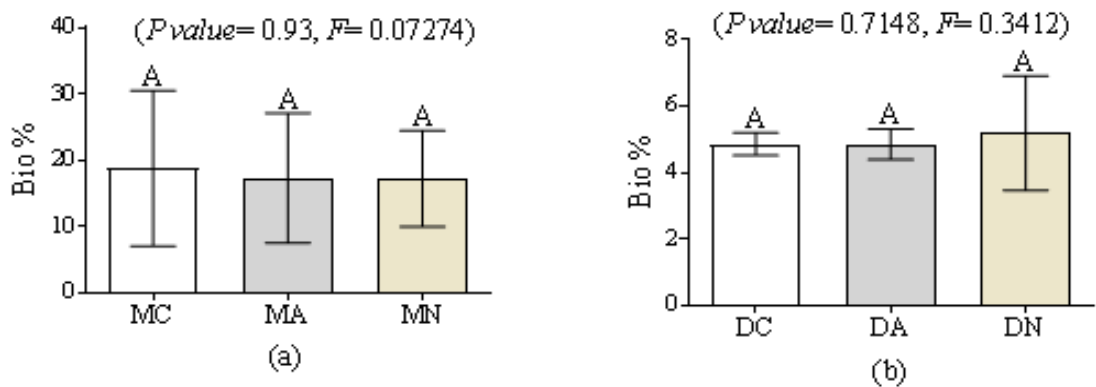


Figure. 14. Biomasses analysis result for mother ramets and daughter ramets, Mother ramets (MC) and daughter ramet (DC) in control treatment, mother ramets (MA) and daughter ramets (DA) in adding fertilizer to mother ramets treatment and (MN) mother ramets and daughter ramets (DN) in adding fertilizer to daughter ramets treatment.

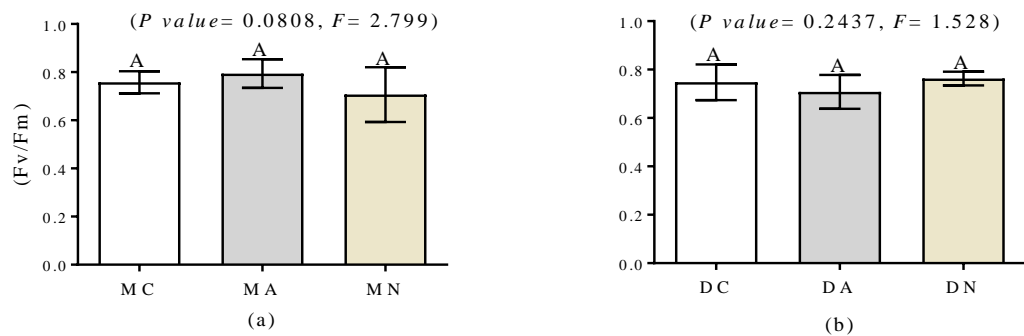


Figure. 15a. Maximum quantum yield (Fv/Fm) of mother ramets at dark condition, (MC) denotes mother under control treatment, (MA) mother with fertilizer and (MN) mother in touch with daughter;  $n$  = 8,  $df$  = 7, Anova analysis.

Figure. 15b. Maximum quantum yield (Fv/Fm) of daughter ramets at dark condition, (DC) denotes daughter under control treatment, (MA) daughter in touch with mother and (MN) daughter with fertilizer;  $n$  = 8,  $df$  = 7, Anova analysis.

The chlorophyll Fv/Fm analysis for mother and daughter ramets did not show any effect of nutrient addition in mother ramets ( $P$  value = 0.0808,  $F$  = 2.799) (Fig. 15a) and daughter ramets ( $P$  value = 0.2437,  $F$  = 1.528) (Fig. 15b).

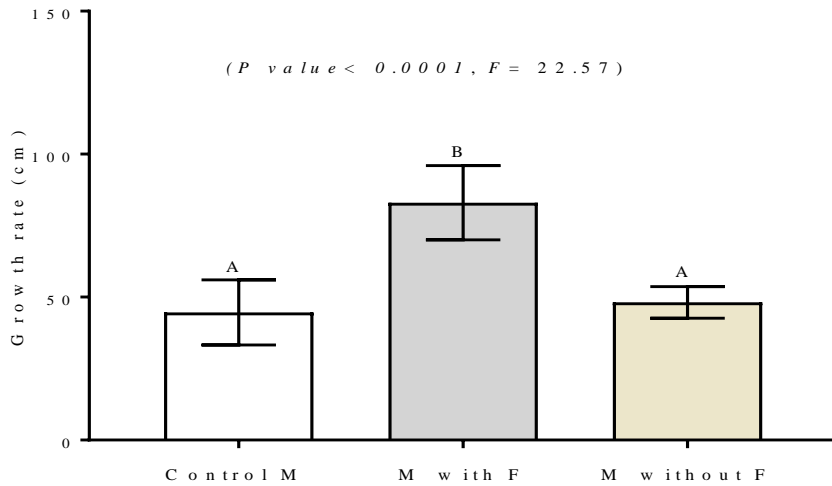


Figure. 16. Growth rate of mother ramets in three treatment (1. Control treatment (Control M), 2. Adding fertilizer to mother ramets (M with F) and 3. Adding fertilizer to daughter ramets (M without F)).

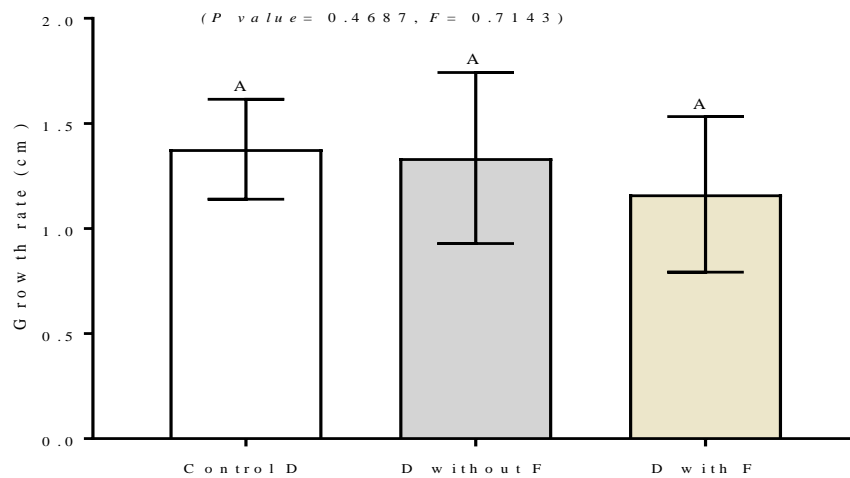


Figure. 17. Growth rate of daughter ramets in three treatment (1. Control treatment (Control D), 2. Adding fertilizer to mother ramets (D without F) and 3. Adding fertilizer to daughter ramets (D with F)).

The growth rate for mother ramets showed a significant difference ( $P$  value < 0.0001,  $F$  = 22.57). Mother ramets had higher growth rates when added a fertilizer (Fig. 16). On the other hand, daughter ramets did not showed any significant effect of fertilizer addition ( $P$  value = 0.4687,  $F$  = 0.7143) (Fig. 17).

## Discussion

In this study, I added a fertiliser to the soil of both mother and daughter ramets in three treatments. First treatment, while the mother ramets were connected the daughter ramets, Second added fertilizer to daughter ramets while connected mother ramets, third added fertilizer to daughter ramets while disconnected mother ramets. Separating the stolon causes death of all ramets and the whole stolon (personal observation). Even though, under water and nutrient shallow and small roots of a new ramets is not able to take nutrient and water independently, this suggestion consistent with He *et al.* (2012) who finds the ramets experienced physiological stress due to reduced water supply from mother ramets.

Clonal integration behaviour might vary among clonal plants (Wolfer and Straile, 2012). When the rhizome in *P. villosa* was separated, the rhizome length and number were reduced, and the node number was reduced as well during the experiment (Dong and Alaten, 1999). Also, the competitive ability against neighbouring plants was reduced in *Solidago canadensis* (Hartnett and Bazzaz, 1985; Peltzer, 2002), the speed of growth decreased (Pennings and Callaway, 2000; Peltzer, 2002), and the number of leaves and rhizomes decreased (Yu *et al.*, 2010). In *O. compressa* all the new ramets died when the stolon was cut between mother and daughter ramets, even those with well-established root systems (Personal observation). This result indicates that the connection between mother and daughter ramets is essential to ensure that the new ramets receive enough resources for a prolonged period to allow them to survive in this harsh environment (de Kroon and Knops, 1990; Cain, 1994; Evans and Cain, 1995; Brewer and Bertness, 1996; Stuefer *et al.*, 1996; Charpentier *et al.*, 1998; Stoll *et al.*, 1998; Pennings and Callaway, 2000). I expect that the dependence of daughter ramets in these harsh environmental conditions is much more important not only to improve the ramets' growth and resistance

to stress, but to secure survivorship in the dry season (Hartnett and Bazzaz, 1985; Jónsdóttir and Callaghan 1990; Alpert 1991; Evans, 1991, 1992; Stuefer *et al.*, 1996; Pennings and Callaway, 2000).

The results of the analysis of the nutrients in both mother and daughter ramets when untreated did not show any significant difference. The reason for that might be the efficiency of mother ramets in the distribution of nutrients between ramets and stolons. However, when I added a fertiliser to the mother ramets, the daughter ramets recorded significantly higher concentrations than mother ramets, possibly because the mother ramets were still supplying daughters with nutrient. Contrarily, daughters that received nutrient did retain them. This reinforces the view that even under conditions where growth is not happening (because of severe water limitation most likely) the dominant strategy is to accumulate nutrients in young ramets.

The amount of resources available and the difference between the levels in patches determine the plant growth, resulting in the distribution of resources between ramets connected in different patches (Alpert, 1999; Alpert *et al.*, 2003; Nilsson and D'Hertefeldt, 2008; He *et al.*, 2011). The movement of nutrients normally goes from the oldest (the mother) to the newest (new ramets) as seen in *O. compressa* in this study and in other plants (Slade and Hutchings, 1987; Caraco and Kelly, 1991; Alpert, 1999), but unlike other studies, the movement would not be reversed from daughter ramets to mother ramets (Peltzer, 2002). Furthermore, the new ramets have limited roots systems. In spite of this, when I added a fertiliser to daughter ramets the concentration of nutrients recorded in daughters was higher than in mothers. This indicates that in spite of the limited root system and the low water availability in the soil the daughter ramets were able to absorb the nutrients, indicating that they are able to provide, at least partially, to their own mineral nutrition. According to (He *et al.*, 2011), the vegetative parts of daughter ramets

remain linked to the mother ramets until a substantial root system has been established to allow them to become independent. Unfortunately, I do not have enough information yet on when daughter ramets of *O. compressa* begin to depend on themselves. Sectioning stolons connecting daughter ramets of different sizes is warranted to provide critical information to this respect.

When fertiliser was added to mother ramets, there was no significant difference in the concentration of nutrients compared with the control treatment that did not have any added nutrients, but they continued to grow and produce new stolons and ramets. Thus, the mother ramets' ability to support new ramets depends on the conditions. For example, the *Potamogeton perfoliatus* mother ramets that got enough light and nutrition continued to support new ramets, but those grown in shady locations stopped supporting new ramets (Wolfer and Straile, 2012).

When nutrients are available underneath a plant, the plant will increase its photosynthesis and grow in biomass unless other factors act as limiting to growth (Blair and Perfecto, 2004; He *et al.*, 2011). However, when I added nutrients to the soil under mother and daughter ramets, it did not affect the size of the biomass or the speed of growth of new ramets (Yu *et al.*, 2010). According to the theory of effort division in the clonal plant system (Hutchings and Wijesinghe, 1997; Wolfer and Straile, 2012), ramets might continue to absorb nutrients, depending on the aggregate root system, if they are unable to perform photosynthesis (Jónsdóttir and Callaghan, 1990; Wolfer and Straile, 2012). When I cut the stolons between mother and daughter ramets, this caused to stop the supply of nutrient and water from mother ramets and because of their inability to absorb sufficient nutrients and water from the soil, they died.

In addition, the addition of nutrients for mother lead to increase the growth rate compared to the control treatment, which did not had no extra nutrients. This might be caused the

large size of the mother's root system, which improved the above ground tissue for mother ramets (personal observation) and also, mother ramets continued to produce more ramets with continued to support the current daughter ramets. On the other hand, daughter ramets did not benefited from the addition of nutrients around it which might be due to the daughter ramets roots system being too small to absorb nutrients effectively. Root systems of these ramets were no more than 1.5 cm in most of daughter ramets I collected. In this study I concluded that the mother ramets of *O. compressa* support the new ramets they produce by providing carbohydrates, nutrients, and water through the stolon, depending on the available nutrients in the habitat.

## References

- Al Musallam, MS** 2007, 'Effect of Protection on Vegetation and Soil Seed Bank in Thumama Area' MA thesis, King Saud University, Riyadh.
- Alpert, P & Mooney, H** 1986. 'Resource sharing among ramets in the clonal herb, *Fragaria chiloensis*', *Oecologia*, vol. 70, no. 2, pp. 227-233.
- Alpert, P** 1999. 'Clonal integration in *Fragaria chiloensis* differs between populations: ramets from grassland are selfish', *Oecologia*, vol. 120, no. 1, pp. 69-76.
- Alpert, P** 1991. 'Nitrogen sharing among ramets increases clonal growth in *Fragaria chiloensis*'. *Ecology*, vol. 72, no. 1, pp. 69-80.
- Alpert, P., Holzapfel, C. & Slominski, C** 2003. 'Differences in performance between genotypes of *Fragaria chiloensis* with different degrees of resource sharing'. *Journal of Ecology*, vol. 91, no. 1, pp. 27-35.
- Bartels S & Chen HY** 2010. 'Is understory plant species diversity driven by resource quantity or resource heterogeneity?', *Ecology*, vol. 91, no. 7, pp. 1931-1938.

- Blair BC & Perfecto I** 2004. 'Successional status and root foraging for phosphorus in seven tropical tree species', *Canadian journal of forest research*, vol. 34, no. 5, pp. 1128-1135.
- Bremner, J.M & Mulvaney, C.S** 1982. 'Methods of soil analysis', part 2 chemical and Micro biological properties, 595-624.
- Brewer, JS & Bertness, MD** 1996, 'Disturbance and intraspecific variation in the clonal morphology of salt marsh perennials', *Oikos*, pp. 107-116.
- Cain, ML** 1994, 'Consequences of foraging in clonal plant species', *Ecology*, vol. 75, no. 4, pp. 933-944.
- Caraco, T & Kelly, CK** 1991, 'On the Adaptive Value of Physiological Integration in Colonial Plants', *Ecology*, pp. 81-93.
- Charpentier, A, Mesleard, F & Thompson, J** 1998, 'The effects of rhizome severing on the clonal growth and clonal architecture of *Scirpus maritimus*', *Oikos*, pp. 107-116.
- Collenette, S** 1999. Wildflowers of Saudi Arabia, National Commission for Wildlife Conservation and Development (NCWCD), Riyadh.
- de Kroons, H & Hutchings, MJ** 1995, 'Morphological plasticity in clonal plants: the foraging concept reconsidered', *Journal of Ecology*, pp. 143-152.
- de Kroon, H & Knops, J** 1990, 'Habitat exploration through morphological plasticity in two chalk grassland perennials', *Oikos*, pp. 39-49.
- Dong, M & Alaten, B** 1999. 'Clonal plasticity in response to rhizome severing and heterogeneous resource supply in the rhizomatous grass *Psammodloa villosa* in an Inner Mongolian dune, China', *Plant Ecology*, vol. 141, no. 1-2, pp. 53-58.
- Evans, JP** 1991, 'The effect of resource integration on fitness related traits in a clonal dune perennial, *Hydrocotyle bonariensis*', *Oecologia*, vol. 86, no. 2, pp. 268-275.

- Evans, JP** 1992, 'The effect of local resource availability and clonal integration on ramet functional morphology in *Hydrocotyle bonariensis*', *Oecologia*, vol. 89, no. 2, pp. 265-276.
- Evans, JP & Cain, ML** 1995, 'A spatially explicit test of foraging behavior in a clonal plant', *Ecology*, pp. 1147-1155.
- Hartnett, D & Bazzaz, F** 1985, 'The integration of neighbourhood effects by clonal genets in *Solidago canadensis*', *The Journal of Ecology*, pp. 415-427.
- He, WM, Alpert, P, Yu, FH, Zhang, LL & Dong, M** 2011, 'Reciprocal and coincident patchiness of multiple resources differentially affect benefits of clonal integration in two perennial plants', *Journal of Ecology*, vol. 99, no. 5, pp. 1202-1210.
- He, W-M, Shen, Y & Cornelissen, JHC** 2012, 'Soil nutrient patchiness and plant genotypes interact on the production potential and decomposition of root and shoot litter: evidence from short-term laboratory experiments with *Triticum aestivum*', *Plant and soil*, vol. 353, no. 1-2, pp. 145-154.
- Hutchings, MJ & Wijesinghe, DK** 1997, 'Patchy habitats, division of labour and growth dividends in clonal plants', *Trends in Ecology & Evolution*, vol. 12, no. 10, pp. 390-394.
- Jónsdóttir, IS & Callaghan, TV** 1990, 'Intraclonal translocation of ammonium and nitrate nitrogen in *Carex bigelowii* Torr. ex Schwein. using <sup>15</sup>N and nitrate reductase assays', *New Phytologist*, vol. 114, no. 3, pp. 419-428.
- Luo, W & Zhao, W** 2015, 'Effects of wind erosion and sand burial on growth and reproduction of a clonal shrub', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 217, pp. 164-169.
- Mandavilla, J. P** 1990. 'flora of Eastern Saudi Arabia', Kegan Paul International Limited, London.



- Mommer, L, Visser, EJ, van Ruijven, J, de Caluwe, H, Pierik, R & de Kroon, H** 2011, 'Contrasting root behaviour in two grass species: a test of functionality in dynamic heterogeneous conditions', *Plant and soil*, vol. 344, no. 1-2, p. 347.
- Nilsson, J & D'Hertefeldt, T** 2008, 'Origin matters for level of resource sharing in the clonal herb *Aegopodium podagraria*', *Evolutionary Ecology*, vol. 22, no. 3, pp. 437-448.
- Olsem, S.R & Sommers, L.E** 1982. Phosphorus. In: A.L. Page, R.H. Miller (Eds). *Methods of Soil Analysis. Part 2.* 2nd ed. Agronomy Monograph 9, ASA and SSSA, Madison, WI, pp. 403-430.
- OS-30p+ / Worldwide Trade Thai** n.d., Worldwide Trade Thai, viewed 5 August 2017, <<http://www.worldwidetradethai.com/?product=os-30p>>.
- Peltzer, DA** 2002. 'Does clonal integration improve competitive ability? A test using aspen (*Populus tremuloides* [Salicaceae]) invasion into prairie', *American journal of botany*, vol. 89, no. 3, pp. 494-499.
- Pennings, SC & Callaway, RM** 2000. 'The advantages of clonal integration under different ecological conditions: a community-wide test', *Ecology*, vol. 81, no. 3, pp. 709-716.
- Slade, A & Hutchings, M** 1987. 'An analysis of the costs and benefits of physiological integration between ramets in the clonal perennial herb *Glechoma hederacea*', *Oecologia*, vol. 73, no. 3, pp. 425-431.
- Stoll, P, Egli, P & Schmid, B** 1998, 'Plant foraging and rhizome growth patterns of *Solidago altissima* in response to mowing and fertilizer application', *Journal of Ecology*, vol. 86, no. 2, pp. 341-354.

- Stueffer, J, De Kroon, H & During, H** 1996, 'Exploitation of environmental heterogeneity by spatial division of labor in a clonal plant', *Functional Ecology*, pp. 328-334.
- Wolfer, SR & Straile, D** 2012, 'To share or not to share: clonal integration in a submerged macrophyte in response to light stress', *Hydrobiologia*, vol. 684, no. 1, pp. 261-269.
- Yu, F, Dong, M & Krüsi, B** 2004. 'Clonal integration helps *Psammochloa villosa* survive sand burial in an inland dune', *New Phytologist*, vol. 162, no. 3, pp. 697-704.
- Yu, F-H, Wang, N, He, W-M & Dong, M** 2010. 'Effects of clonal integration on species composition and biomass of sand dune communities', *Journal of Arid Environments*, vol. 74, no. 6, pp. 632-637.

## Chapter 4

# The ability of mother ramets to supply ramets and expansion of stolons under harsh environmental conditions.

### Introduction

Using clonal integration, some plants can reproduce asexually by producing rhizomes or stolons that generate new ramets. While initially the daughter ramets are connected to the mother ramet and completely depend on it, when they grow and mature they can become standalone plants that can eventually produce more leaves, roots, rhizomes and stolons (Walfer and Straile, 2012). Initially mother ramets produce a new daughter ramet (recipient) and supply them with water, nutrients and carbohydrates via stolon or rhizomes (Alpert and Mooney, 1986; de Kroon and Hutchings, 1995; Peltzer, 2002; Walfer and Straile, 2012). Individual ramets existing on stolons can share water, nutrients and carbohydrates with mother ramets (Peltzer, 2002). Clonal integration enables ramets to establish under harsh environmental conditions (Salzman and Parker, 1985; Slade and Hutchings, 1987; Pennings and Callaway, 2000; Peltzer, 2002) but can also allow the genet to efficiently exploit soil resources (de Kroon and Knops, 1990; Evans and Cain, 1995; Peltzer, 2002), enhancing their chances of survival and reducing the effects of competition for individual ramets (Stuefer *et al.*, 1996; Alpert, 1999; Amsberry *et al.*, 2000; Pennings and Callaway, 2000; D'Hertefeldt and Falkengren-Grerup, 2002; Peltzer, 2002; Yu *et al.*, 2008; Luo and Zhao, 2015) and enhances growth in heterogeneous environments (Stuefer *et al.*, 1994; de Kroon and Hutchings, 1995)

The clonal integration mechanism is one of the most important ways for some plants to resist harsh environmental conditions (Peltzer, 2002) and to search for resources and

nutrients in patchy and unpredictable environments (de Kroon and Hutchings, 1995). The clonal integration mechanism is considered very useful for invasive plants by allowing them to dominant of the area (Pennings and Callaway, 2000; Peltzer, 2002), and some research suggests that the exchange of resources and nutrients allows individual ramets to spread quickly and exploit the soil's resources in patches (de Kroon and Knops, 1990; Cain, 1994; Evans and Cain, 1995; Shumway, 1995; Peltzer, 2002). A study by Wolfer and Straile (2012) found that clonal plants usually take advantage of this mechanism in resource poor areas. It is common and widespread in sandy environments, where ramets share resources over long periods (Wolfer and Straile, 2012). However, Wolfer and Strail (2012) found that there is no one way direction of nutrient movement between mother and daughter ramets, which seems contrary to what I documented in the previous chapter which is that nutrients move from mother ramets to daughter ramets in *O. compressa*. In some resource rich habitats, clonal plants aim to produce more ramets to increase their absorption of water and nutrients (de Kroon and Hutchings, 1995; Evans and Cain, 1995; Dong *et al.*, 1997; Oborny *et al.*, 2012; Martina and Von Ende, 2013).

The competition for nutrients and resources is one of the environmental conditions that threaten the growth of plants and the spread of daughter ramets in poor environments (Penning and Callaway, 2000). Sharing resources, water and carbohydrates with the daughter ramets, contributes to reduce the impact of competition from other plants on clonal plants (Peltzer, 2002). Clonal integration thus allows spreading and expanding into open areas, as a strategy to resist competition on resources and resistance of invasive plants (de Kroon and Knops, 1990).

Clonal plant resistance to competition need further research and study. The information is still insufficient to understand clonal plants behaviour, also the impact of clonal integration on resistance of competition (Wang *et al.*, 2008). In this regard, some

researchers have recorded that clonal integration has a role in resistance of competition through increasing the spread and expansion (Dong and Alaten, 1999; Cabaço *et al.*, 2013) and the exploitation of soil resources (Peltzer, 2002; Ravi *et al.*, 2008; Yan *et al.*, 2013), to dominance on open areas (Wang *et al.*, 2008; Xiao *et al.*, 2011). In addition, there is a potential for improved the clonal integration to resistance of clonal plant for competition for individual ramets, and resistance of the stress conditions (Pennings and Callaway, 2000), which might be cause to reduce the growth and reproduction of neighboring plants (Wang *et al.*, 2008). On the other hand, some studied have indicated that clonal integration does not improve the clonal integration resistance to competition (Schmid and Bazzaz, 1987; Amsberry *et al.*, 2000; Peltzer, 2002). Hence, the spreading and expansion does not mean taking advantage of soil resources and absorption from soil, which might be cause to accumulate the nutrients and resources in soil (Dong and Alaten, 1999).

In this research, I seek to understand the efficiency of support mother ramets provide to daughter ramets through distance between both of them, by measuring the concentration of nitrogen, phosphorus and potassium in both mother and daughter ramets, as well as through photosynthesis efficiency in both mother and daughter ramets. I assume that when the distance between mother and daughter ramets is shorter that encourage mother ramets to increase supporting for daughter ramets.

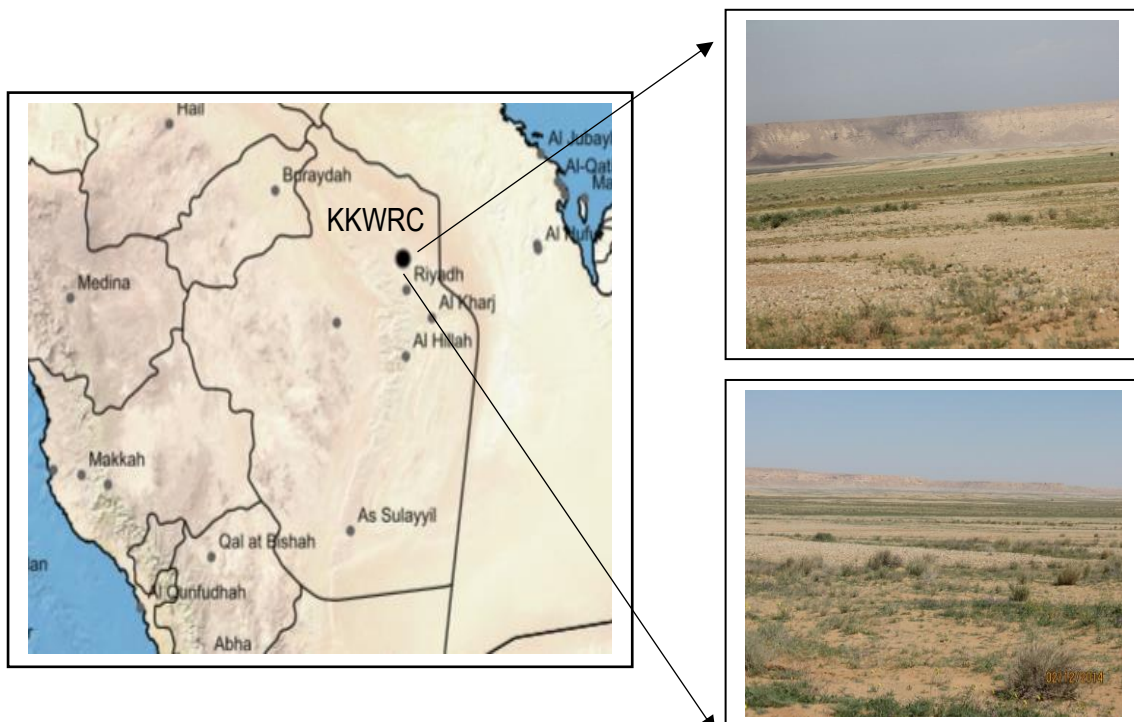
## Materials and methods

### *Study area*

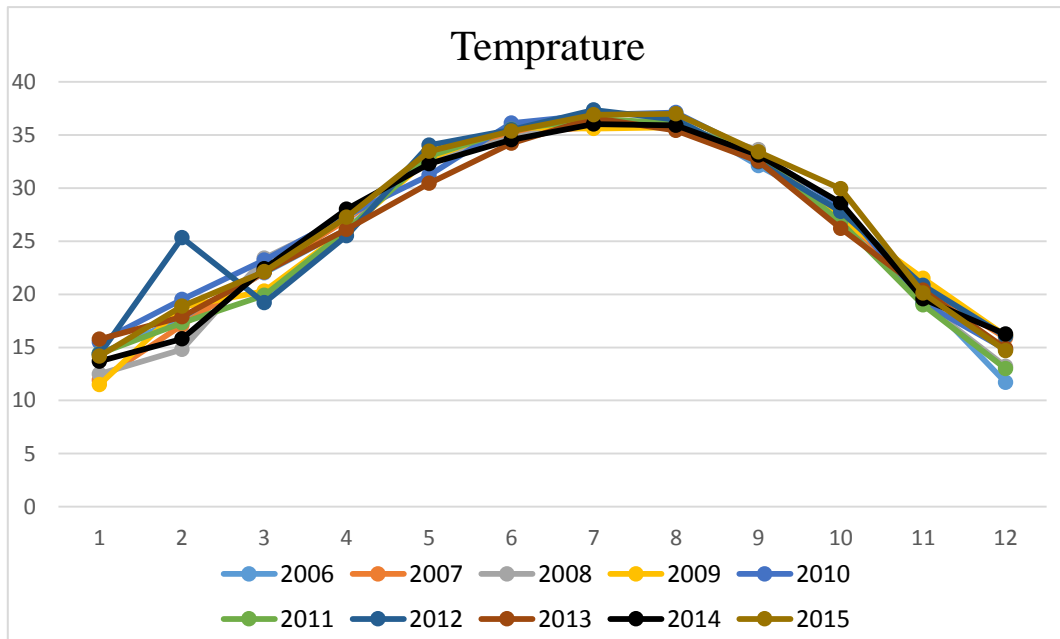
The study field area, King Khalid Wildlife Research Centre (KKWRC) in the Al-Thumamah area, is protected by the Saudi Arabian government. The Al-Thumamah area (N 25°11'8", E 46°38'2") is about 70 km from Riyadh city (Fig. 1), at an altitude of approximately 567 m and covers an area of approximately 170 km<sup>2</sup>. The Al-Thumamah

area consists mostly of sandy soil with rocks and gravelly soil (Al Musallam, 2007). Its vegetation contains shrubs, perennial and annual plants.

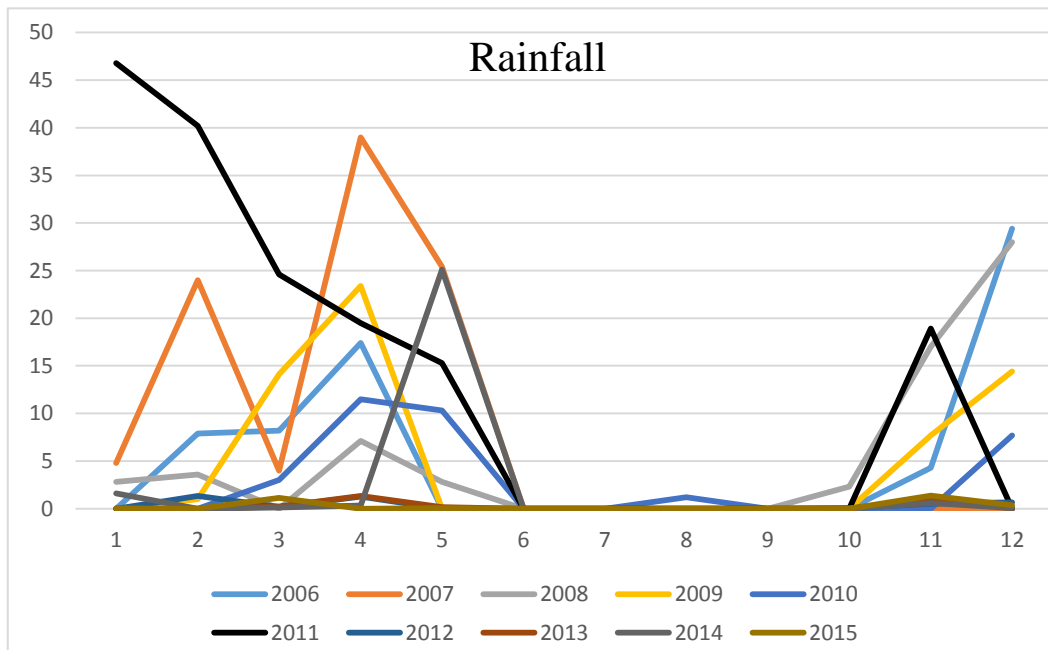
The study area is characterized by a dry climate: the average temperature in summer is 38 °C and during winter 12 °C (Fig. 2 a). Furthermore, the rainfall is less than 100 mm annually on average all falling between October and May (The General Authority of Meteorology and Environmental Protection) (Fig. 2 b).



(Figure. 1. The left picture for the KKWRC area at Saudi Arabia map, and in right pictures for study sites).



(Figure. 2 a. The temperature rate among 2006 to 2015.)

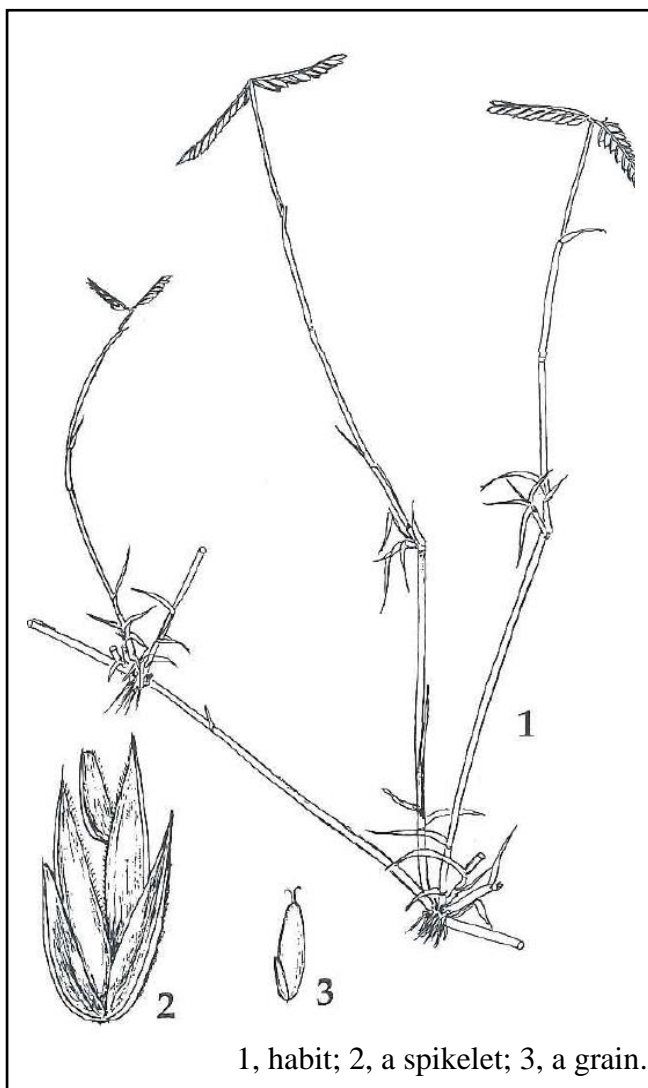


(Figure. 2 b. The rate monthly rainfall over ten years from 2006 to 2015).

### *Plant description*

*Ochthochloa compressa* is a perennial native grass in the Arabian Peninsula. It has two mechanisms of reproduction: sexually by producing seeds and spikes and

asexually by producing stolons. Adult ramets (mothers) reach up to 70 cm in height (Collenette, 1999), and stolons may extend up to 150 cm (personal observation). The stolons are almost 3.7 mm diameter on average, and typically there are 5 nodes, each supporting one ramets (Fig. 3). It produces seeds in February and March (personal observation). *O. Compressa* is widespread in the Arabian Peninsula (Mandavilla, 1990; Collenette, 1999), the western and dry tropical regions of Asia, and the northwest dry tropical regions of India and Africa.



(*Ochthochloa compressa* - Flora of Qatar 2016).



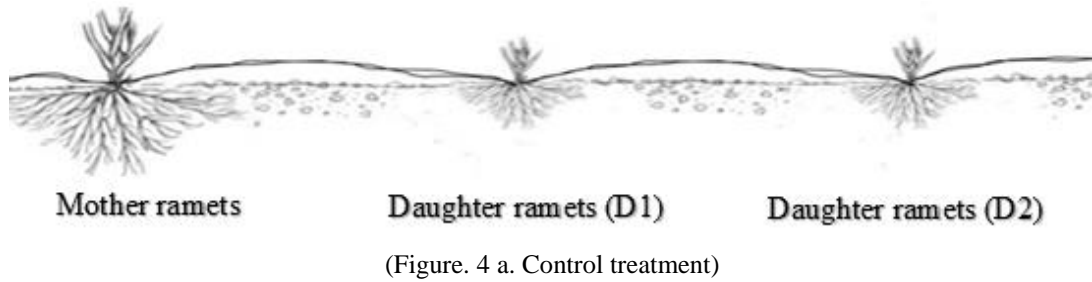
(Figure. 3. Left picture for morphological shape for *Ochthochloa compressa* (Collenette, 1999), and right picture for spikes.)



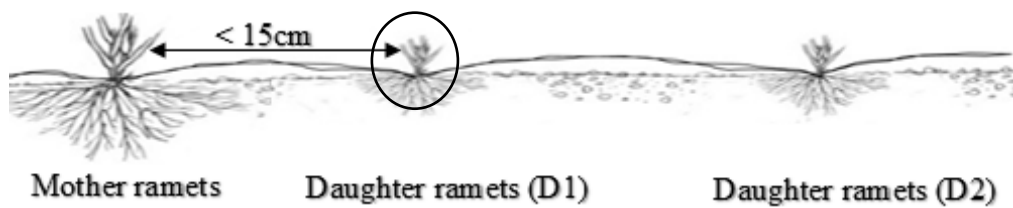
## Data collection

I designed our experiment with four treatments as follows:

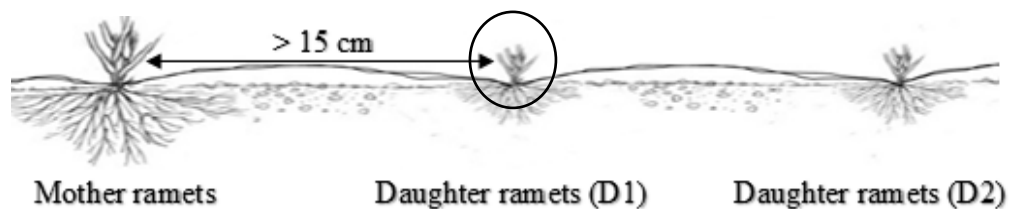
1. Control treatment of mother and daughter ramets were selected randomly (Fig. 4a).



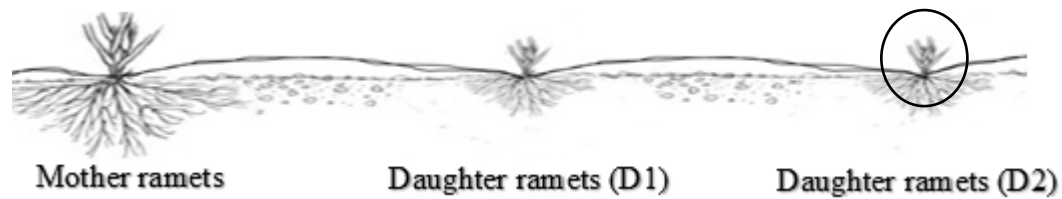
2. Daughter ramets separated by less than 15cm from the mother ramets (Fig. 4 b).



3. Daughter ramets separated by more than 15 cm from the mother ramets (Fig. 5 c).



4. Second daughter ramets were randomly chosen (Fig. 4 d).



(Figure. 4 d. Second daughter ramet's treatment)

In the last week in March 2014, I collected our samples to study how distance between mother and daughter ramets affected the performance of the young ramets, an important component of clonal integration in *O. compressa* to resistance of competition, also effect of that on the relation between mother and daughter ramets depending on the distance between mother and daughter ramets. I focused on nitrogen, phosphorus and potassium concentrations in both mother and daughter ramets to understand the effect of competition on both of them. I measured the efficiency of photosynthesis among in both mother and daughter ramets by OS30p device (Fig. 5) to measure Fv/Fm. Subsequently, I harvested all the above ground material or both mother and daughter ramets to prepare it for drying, grinding and analysing in laboratory at Ministry of Environment Water & Agriculture in Saudi Arabia. I dried the above ground tissue in an electric oven at 60 C° for 48 hours and then grind it. Then, I sent it to laboratory to analyse the concentrations of nitrogen, phosphorus and potassium in both mother and daughter ramets. They followed the Kjeldahl method to estimate the total N (Bremner and Mulvaney, 1982) and estimated the total P and K using the spectrophotometer method (Olsem and Sommers, 1982).



(Figure. 5. OS30p device (OS-30p+ / Worldwide Trade Thai n.d.).

### *Statistical analysis*

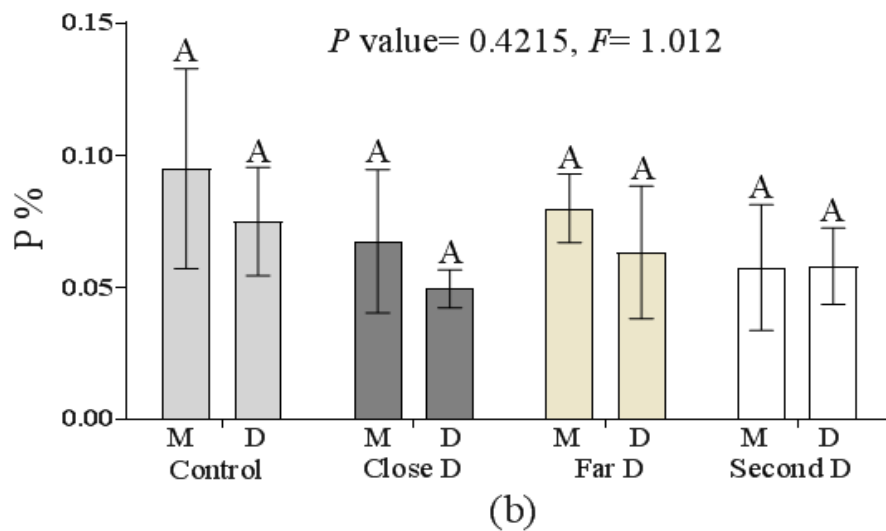
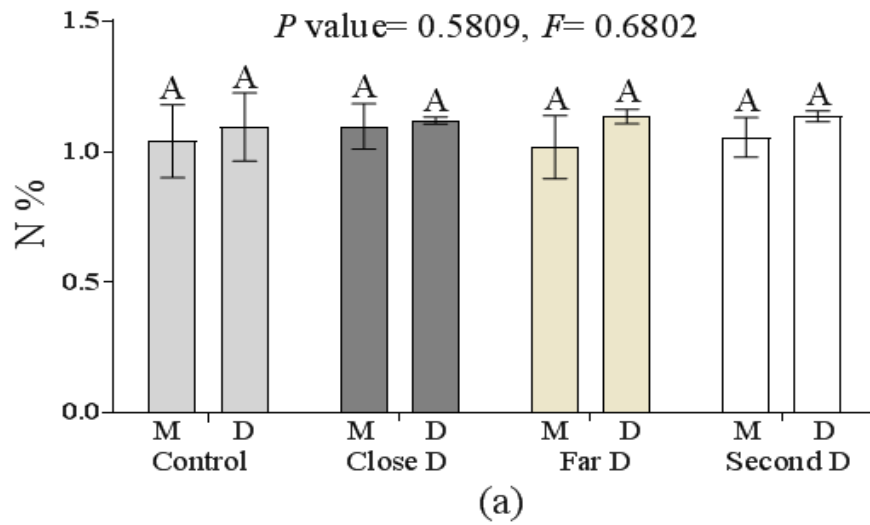
During the diagnosis, our data showed equal variance, and all of the data were normally distributed. I therefore analysed the data using a two-way ANOVA to detect differences in the concentrations of elements (nitrogen, phosphorus and potassium), biomass and photosynthetic efficiency among the mother and daughter ramets. I used this test to determine whether there is a competitive relationship between mother and daughter ramets.

### **Results**

The total amounts of nitrogen in mother and daughter ramets were not significantly different ( $P$  value = 0.5809,  $F$  = 0.6802) (Fig. 6 a).

In the analysis of total phosphorus, I found that there was no significant difference between the mother and daughter ramets in the sample ( $P$  value = 0.4215,  $F$  = 1.012). The control samples recorded the highest concentration of total phosphorus, followed by the close daughter ramets (Fig. 6 b).

On the other hand, there was a significant difference between the potassium content of mother and daughter ramets, ( $P < 0.0001$ ,  $F= 1.211$ ) (Fig. 6 c). The control samples showed the highest concentration of total potassium compared with the other samples, and there was no significant difference between the control mother and daughter ramets.



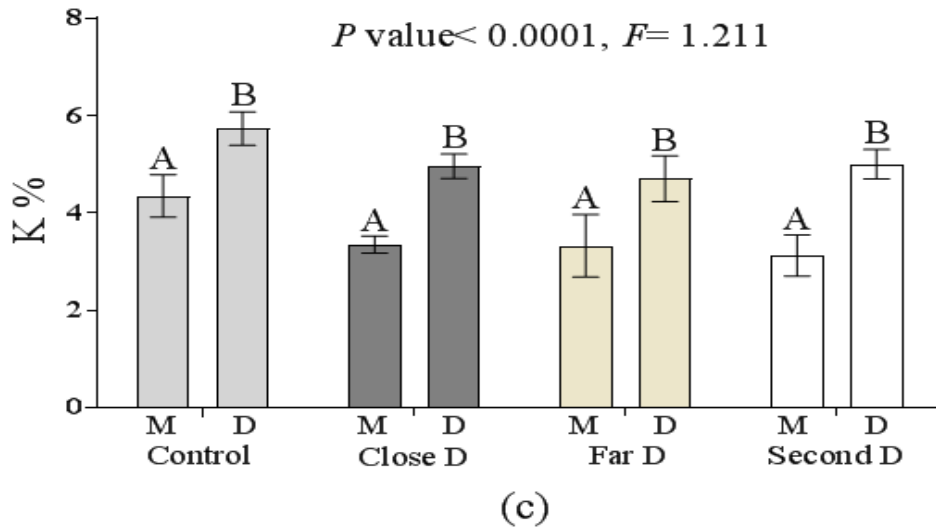


Figure. 6. Content of nitrogen (a), phosphours (b) and ppotassium (c) concentration in mother ramets and daughter ramets among (Control, Close (less than 15cm), Far (More than 15cm) and second daughter).

The analysis of photosynthetic efficiency showed no significant difference between the mother and daughter sample ramets ( $P \text{ value} = 0.6264, F \text{ value} = 0.5905$ ) (Fig. 7). The results were generally close or almost the same, and there were no significant differences between the samples.

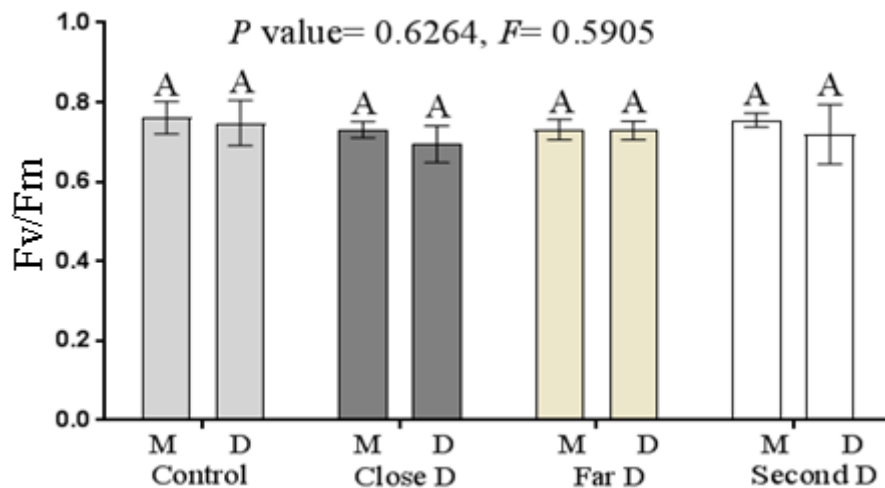


Figure. 7. Content the amount of efficiency of photosynthesis in mother ramets and daughter ramets among (Control, Close (less than 15cm), Far (More than 15cm) and second daughter).

## Discussion

Our results of nitrogen and phosphorus analysis showed no significant differences between mother ramets and daughter ramets, suggesting that mother to daughter ramets transfer was not affected by the distance between them indicating that distance between mother and daughter has no effect on daughter performance. This might be because the priority strategy of mother ramets to provide nutrients, carbohydrate, water and elements for daughter ramets (Martina and Von Ende, 2013). The relative reduction in nitrogen and phosphorus of daughter ramets may indicate the daughter ramets do not need to accumulate those elements. In addition, it suggests that this enhances the ability of *O. compressa* to cope with competition from neighboring plants by producing stolons to spread that are highly subsidized. This finding suggest that clonal integration improves the competitiveness of ramets (de Kroon and Schieving, 1991), and hence reduces the impact of competition in dense patches (Xiao *et al.*, 2011). Unlike our results Martina and Von Ende (2013) recorded that increased the distance between mother ramets and daughter ramets reduced the competition between them, and that the clonal integration does not improve the competitiveness for clonal plants (see also Schmid and Bazzaz, 1987; de Kroon and Kalliola, 1995; Amsberry *et al.*, 2000; Peltzer, 2002; Pennings and Callaway, 2000).

In contrast to nitrogen and phosphorus, the results of potassium analysis showed significant differences between mother ramets and daughter ramets. All daughter ramets had higher concentration of potassium in above ground tissue than mother ramets. Increasing the concentration of potassium in a daughter might be a strategy of ramets to enhance the ability of ramets to resist the harsh environmental conditions by securing strict stomatal control (Wang *et al.*, 2013) and enzyme activation (Cakmak, 2005). Also, the daughter ramets may accumulate potassium to continue of growth and enhance of the

photosynthetic mechanism either by helping to maintain stomatal control, or contributing to osmotic regulation that allows continuous flow of water via a steep water potential gradient (Cakmak, 2005; Wang *et al.*, 2013). In addition, this might be attributed to protect of chloroplasts from oxidative damage due to drought and for water relations within the plant (Cakmak, 2005).

There were no significant differences in the efficiency of photosynthesis between mother ramets and daughter ramets, which is a good indicator of the ability of the *O. compressa* species to adapt to harsh environmental conditions such as those in our study area. Indeed the values recorded are quite high and suggest that the photosynthetic apparatus is not affected by the dry prevalent conditions. This is also the case for the daughter ramets, in spite of their very shallow roots (personal observation) that are unable to support them if disconnected from the mother ramet. Water and nutrient supply from the mother ramet must be high enough to prevent chronic photoinhibition. This in turn suggest that the mother probably has deep root systems allowing to reach water stored in lower soil layers.

This research highlights the potential value of *O. compressa* because of its tolerance to stress, being considered as a palatable plant for herbivores. Also, I believe that its ability to grow and adapt to harsh environments can make it a potentially very useful species for restoration of degraded areas. This is because *O. compressa* benefits from the mechanism of clonal integration through the continued support of the mother to the daughter ramets and continues growth during those cases. Thus, our results are important to the understanding of the mechanism of clonal integration in *O. compressa* and the role of this mechanism in the resistance to environmental conditions.

## References

- Al Musallam, MS** 2007, 'Effect of Protection on Vegetation and Soil Seed Bank in Thumama Area' MA thesis, King Saud University, Riyadh.
- Alpert, P & Mooney, H** 1986. 'Resource sharing among ramets in the clonal herb, *Fragaria chiloensis*', *Oecologia*, vol. 70, no. 2, pp. 227-233.
- Alpert, P** 1999, 'Clonal integration in *Fragaria chiloensis* differs between populations: ramets from grassland are selfish', *Oecologia*, vol. 120, no. 1, pp. 69-76.
- Amsberry, L, Baker, M.A, Ewanchuk, P.J & Bertness, M.D** 2000, 'Clonal integration and the expansion of *Phragmites australis*', *Ecological applications*, vol. 10, no. 4, pp. 1110-1118.
- Bremner, J.M .,& Mulvaney, C.S** 1982. Methods of soil analysis, part 2 chemical and Micro biological properties, 595-624.
- Cabaço, S, Apostolaki, ET, García-Marín, P, Gruber, R, Hernandez, I, Martínez-Crego, B, Mascaro, O, Perez, M, Prathep, A & Robinson, C** 2013. 'Effects of nutrient enrichment on seagrass population dynamics: evidence and synthesis from the biomass–density relationships', *Journal of Ecology*, vol. 101, no. 6, pp. 1552-1562.
- Cain, ML** 1994, 'Consequences of foraging in clonal plant species', *Ecology*, vol. 75, no. 4, pp. 933-944.
- Cakmak, I** 2005. 'The role of potassium in alleviating detrimental effects of abiotic stresses in plants', *Journal of Plant Nutrition and Soil Science*, vol. 168, no. 4, pp. 521-530.
- Collenette, S** 1999. 'Wildflowers of Saudi Arabia', National Commission for Wildlife Conservation and Development (NCWCD), Riyadh.



- D'Hertefeldt, T & Falkengren-Grerup, U** 2002, 'Extensive physiological integration in *Carex arenaria* and *Carex disticha* in relation to potassium and water availability', *New Phytologist*, vol. 156, no. 3, pp. 469-477.
- de Kroon, H & Kalliola, R** 1995, 'Shoot dynamics of the giant grass *Gynerium sagittatum* in Peruvian Amazon floodplains, a clonal plant that does show self-thinning', *Oecologia*, vol. 101, no. 1, pp. 124-131.
- de Kroon, H & Knops, J** 1990. 'Habitat exploration through morphological plasticity in two chalk grassland perennials', *Oikos*, pp. 39-49.
- de Kroon, H & Schieving, F** 1991. 'Resource allocation patterns as a function of clonal morphology: a general model applied to a foraging clonal plant', *The Journal of Ecology*, pp. 519-530.
- de Kroon, H & Hutchings, MJ** 1995. 'Morphological plasticity in clonal plants: the foraging concept reconsidered', *Journal of Ecology*, pp. 143-152.
- Dong M, During H, & Werger M** 1997. Clonal plasticity in response to nutrient availability in the pseudoannual herb, *Trientalis europaea* L. *Plant Ecology*, vol. 131, no. 2, pp. 233-239.
- Dong, M & Alaten, B** 1999. 'Clonal plasticity in response to rhizome severing and heterogeneous resource supply in the rhizomatous grass *Psammochloa villosa* in an Inner Mongolian dune, China', *Plant Ecology*, vol. 141, no. 1-2, pp. 53-58.
- Evans, JP & Cain, ML** 1995, 'A spatially explicit test of foraging behavior in a clonal plant', *Ecology*, pp. 1147-1155.
- Luo, W & Zhao, W** 2015. 'Effects of wind erosion and sand burial on growth and reproduction of a clonal shrub', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 217, pp. 164-169.

- Mandavilla, J. P** 1990. 'Flora of Eastern Saudi Arabia', Kegan Paul International Limited, London.
- Martina, J & Von Ende, C** 2013. 'Increased spatial dominance in high nitrogen, saturated soil due to clonal architecture plasticity of the invasive wetland plant, *Phalaris arundinacea*', *Plant Ecology*, vol. 214, no. 12, pp. 1443-1453.
- Oborny B, Mony C &, Herben T** 2012. 'From virtual plants to real communities: a review of modelling clonal growth'. *Ecological Modelling*, vol. 234, pp. 20-30.
- Ochthochloa compressa - Flora of Qatar*** 2016, Flora of Qatar, viewed 6 April 2017, <[http://www.floraofqatar.com/ochthochloa\\_compressa.htm](http://www.floraofqatar.com/ochthochloa_compressa.htm)>.
- Olsem, S.R & Sommers, L.E** 1982. Phosphorus. In: A.L. Page, R.H. Miller (Eds). *Methods of Soil Analysis. Part 2. 2nd ed. Agronomy Monograph 9, ASA and SSSA, Madison, WI, pp. 403-430.*
- OS30p+ Plant Stress Meter*** n.d., ADC BioScientific Ltd., viewed 19 May 2017, <<http://www.adc.co.uk/products/os30p-plant-stress-meter/>>.
- Peltzer, DA** 2002, 'Does clonal integration improve competitive ability? A test using aspen (*Populus tremuloides* [Salicaceae]) invasion into prairie', *American journal of botany*, vol. 89, no. 3, pp. 494-499.
- Pennings, SC & Callaway, RM** 2000. 'The advantages of clonal integration under different ecological conditions: a community-wide test', *Ecology*, vol. 81, no. 3, pp. 709-716.
- Ravi, S, D'Odorico, P, Wang, L & Collins, S** 2008. 'Form and function of grass ring patterns in arid grasslands: the role of abiotic controls', *Oecologia*, vol. 158, no. 3, pp. 545-555.

- Salzman, A. G., & M. A. Parker, M. A** 1985. 'Neighbors ameliorate local salinity stress for a rhizomatous plant in a heterogeneous environment'. *Oecologia*, vol. 65, no. 2, pp. 273-277.
- Schmid, B & Bazzaz, F** 1987. 'Clonal integration and population structure in perennials: effects of severing rhizome connections'. *Ecology* 68: 2016–2022.
- Shumway, SW** 1995. 'Physiological integration among clonal ramets during invasion of disturbance patches in a New England salt marsh'. *Annals of Botany*, vol. 76, no. 3, pp. 225-233.
- Slade, A & Hutchings, M** 1987, 'An analysis of the costs and benefits of physiological integration between ramets in the clonal perennial herb *Glechoma hederacea*', *Oecologia*, vol. 73, no. 3, pp. 425-431.
- Stuefer, JF, During, HJ & de Kroon, H** 1994. 'High benefits of clonal integration in two stoloniferous species, in response to heterogeneous light environments', *Journal of Ecology*, pp. 511-518.
- Stueffer, J, De Kroon, H & During, H** 1996, 'Exploitation of environmental heterogeneity by spatial division of labor in a clonal plant', *Functional Ecology*, pp. 328-334.
- Wang, M, Zheng, Q, Shen, Q & Guo, S** 2013. 'The critical role of potassium in plant stress response', *International journal of molecular sciences*, vol. 14, no. 4, pp. 7370-7390.
- Wang, N, Yu, F-H, Li, P-X, He, W-M, Liu, F-H, Liu, J-M & Dong, M** 2008. 'Clonal integration affects growth, photosynthetic efficiency and biomass allocation, but not the competitive ability, of the alien invasive *Alternanthera philoxeroides* under severe stress', *Annals of Botany*, vol. 101, no. 5, pp. 671-678.

**Wolfer, SR & Straile, D** 2012. 'To share or not to share: clonal integration in a submerged macrophyte in response to light stress', *Hydrobiologia*, vol. 684, no. 1, pp. 261-269.

**Xiao, Y, Tang, J, Qing, H, Zhou, C & An, S** 2011, 'Effects of salinity and clonal integration on growth and sexual reproduction of the invasive grass *Spartina alterniflora*', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 206, no. 8, pp. 736-741.

**Yan, X, Wang, H, Wang, Q & Rudstam, LG** 2013, 'Risk spreading, habitat selection and division of biomass in a submerged clonal plant: Responses to heterogeneous copper pollution', *Environmental pollution*, vol. 174, pp. 114-120.

**Yu, F-H, Wang, N, He, W-M, Chu, Y & Dong, M** 2008. 'Adaptation of rhizome connections in drylands: increasing tolerance of clones to wind erosion', *Annals of Botany*, vol. 102, no. 4, pp. 571-577.

## Chapter 5

# The effects of simulated grazing of mother ramets or daughter ramets

### Introduction

Clonal integration is one of the strategies used by plants to spread and persist in plant communities in poor and heterogeneous habitats (Martina and Von Ende, 2013). Unlike non-clonal plants, clonal plants can increase absorption of nutrients from the soil when resources are scarce or patchily distributed (de Kroon and Knops, 1990; de Kroon and Hutchings, 1995; Evans and Cain, 1995; Dong *et al.*, 1997; Suzuki and Stuefer, 1999; Wilsey, 2002; Wang *et al.*, 2008; Oborny *et al.*, 2012; Martina and Von Ende, 2013). So, one of the main advantages of clonal integration mechanism is to transfer water, nutrients, and carbohydrates from the mother ramet to the daughter ramets through stolons (Stuefer *et al.*, 1994) which enables daughter ramets to establish a new individual community of ramets (Peltzer, 2002).

Usually, clonal plants that use this mechanism tend to live in heterogeneous environments (Suzuki and Stuefer, 1999; Liu *et al.*, 2009), such as poor sandy environments (Brown, 1997; Yu *et al.*, 2008; Luo and Zhao, 2015) and are well adapted to them (de Kroon and Knops, 1990). Clonal integration contributes to their resistance to harsh environmental conditions (Stuefer *et al.*, 1996; Amsberry *et al.*, 2000; Pennings and Callaway, 2000; D'Hertefeldt and Falkengren-Grerup, 2002; Peltzer, 2002; Yu *et al.*, 2004; Wang *et al.*, 2008; Luo and Zhao, 2015) such as drought (He *et al.*, 2011), environmental stress (Stuefer *et al.*, 1994; Peltzer, 2002) and grazing.

Clonal integration has an important role in resistance to grazing (Wilsey, 2002; Liu *et al.*, 2007; Xiao *et al.*, 2010) and increase the ability of plants to tolerate tissue losses (Liu *et*

*al.*, 2009) by buffering clones from the vegetative parts that are lost (Bach, 2000; Liu *et al.*, 2009). The mechanism of buffering can occur through the sharing of plant resources by the ramets, while they are connected by stolons (Alpert and Mooney, 1986; Liu *et al.*, 2007) or through the use of nutrients and carbohydrates stored in the stolons (Liu *et al.*, 2007) allowing the plant to produce a new tissues to replace those removed by grazing (Suzuki and Stuefer, 1999; Liu *et al.*, 2007).

Clonal integration responses can vary depending on the plant species and environment (de Kroon and Knops, 1990; Stuefer *et al.*, 1994; Strauss and Agrawal, 1999; Ferraro and Oosterheld, 2002; Liu *et al.*, 2007), although some studies have indicated that clonal integration has an effect on plant biomass by increase it (Yu *et al.*, 2002; Roiloa *et al.*, 2007; Wang *et al.*, 2008). In the clonal integration mechanism, grazing plants are characterized by the means in which they compensate for lost tissue due to grazing (Liu *et al.*, 2009). This also contributes to grazing resistance (Strauss and Agrawal, 1999; Bach, 2000; Wilsey, 2002; Liu *et al.*, 2009; Wolfer and Straile, 2012), because the grazing is caused by a loss of vegetative parts, which has an effect on photosynthesis efficiency, but that does not mean grazing has an effect on growth (Ferraro and Oosterheld, 2002; Liu *et al.*, 2009).

All patterns of grazing cause disappearance of all, or a large part of, the vegetative tissues depending on the degree of grazing. As a negative effect, grazing cause removal of the apical parts resulting in a decrease in the biomass density, nutrients and energy required for growth of the buds (Wang *et al.*, 2004). Correspondingly, it is considered a catalyst for the activation of the meristematic cells (Zhao *et al.*, 2008), as well as a stimulation for the lateral buds on growth through removal the competition of apexes parts (Wang *et al.*, 2004). In addition, the mother ramets are the main source of resources and nutrients for daughter ramets through exporting them through functional stolons (Alpert, 1996;

Wang *et al.*, 2004; Zhang *et al.*, 2002) to support both growth and survivorship of the damaged parts of the stolons and daughter ramets (Wang *et al.*, 2004). Mother ramets, rather than soil, are the primary source of nitrogen for daughter ramets (Alpert, 1996), and often the concentration of nitrogen in tissues decreases as the distance increases from the mother. In terms of phosphorus and potassium, the soil is the primary source for the daughter ramets (Headley *et al.*, 1988), these two elements are absorbed through the roots and passed through the xylem towards to above ground tissue. Then, transferred through stolons to other ramets (Alpert, 1991). In order to enable the mother ramets and daughter ramets to absorb the phosphorus and potassium from soil, sufficient moisture content is needed in the soil and sum of roots to compensate the deficiency and harmed tissue above the soil (Stueffer *et al.*, 1996).

The mechanism of photosynthesis is dependent on the above ground tissue (Ferraro and Oosterheld, 2002; Wang *et al.*, 2004). It has been identified that on one hand, grazing reduces the number of leaves, leaf area and biomass density (Zhang *et al.*, 2002), but at the same time this does not imply a decrease in growth in some species (Ferraro and Oosterheld, 2002; Liu *et al.*, 2009). On the other hand, it might cause an increase in the activity of photosynthesis in the remnant tissues (Nowak and Caldwell, 1984). With respect to compensatory photosynthesis mechanism, Chapin *et al.* (1993) found that some of grazed species had become faster to compensate for lost tissues than non-grazed species at the same age and photosynthesis rates as well (Strauss and Agrawal, 1999).

Some authors of the previously mentioned studies have suggested that clonal integration enhances the grazing resistance of clonal plants (Belsky, 1986; Suzuki and Stuefer, 1999), but so far, insufficient information on the role of clonal integration in grazing resistance exists, particularly for extreme environments. My objective is to document the ability of *O. compressa* to resist grazing (when simulated by clipping) through the analysis of

concentration of nitrogen, phosphorus and potassium in both mother ramets and daughter ramets, when exposed to clipping by 50-60%. Besides study the effect of clipping on the efficiency of photosynthesis in both mother and daughter ramets at a same conditions.

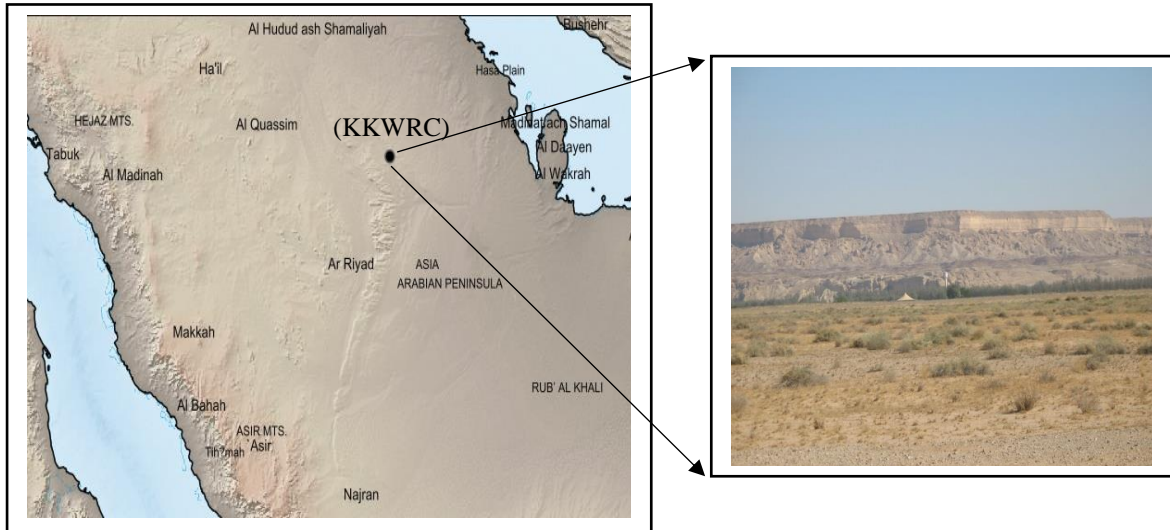
## Material and Methods

### *Study area*

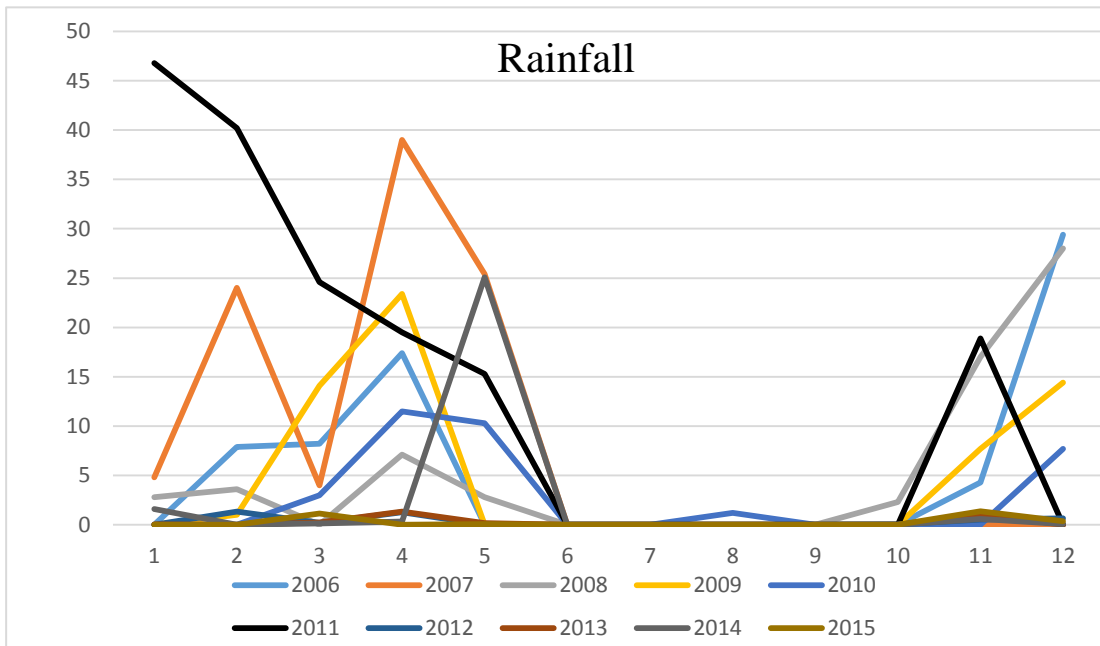
I conducted our experiment in a reserved area of Al- Thumama area (N 25°11'05.43", E 46°41'48.09"), is a located 70 km from Riyadh City at an altitude of approximately 570 m and covering an extension of approximately 170 km<sup>2</sup>. The Al-Thumama area consists mostly of sandy soil with some areas containing exposed rocks and gravelly soil. The grazing considered one of the most common activities in this region, especially a grazing by camels. The climate is dry and the area has average temperatures of 7 °C in the winter and 38 °C in the summer (Al Musallam, 2007) (Fig. 1).

The rainfall usually falls between October and May, and the annual rainfall is almost 100 mm (Fig. 2). Al Thumam area has dry climate and the average temperature in winter is 12 C°; in summer temperatures reach 38 C° (Al Musallam, 2007) (General Authority of Meteorology and Environmental Protection) (Fig. 3).

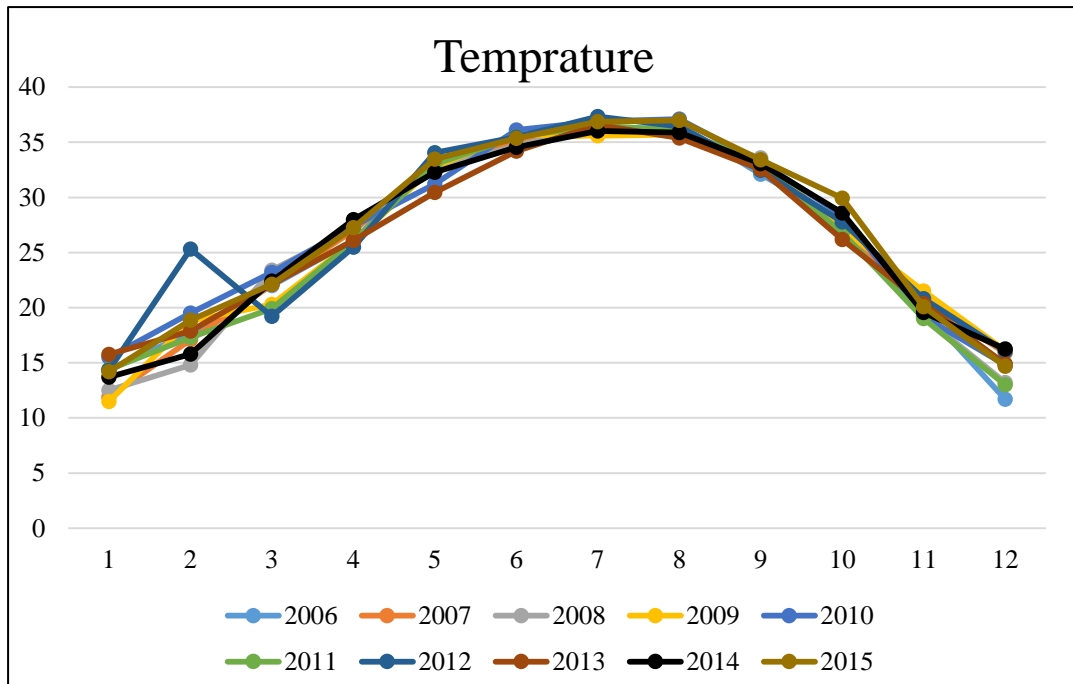




(Figure. 1. The left picture for the King Khalid Wildlife Research Centre (KKWRC) area at Saudi Arabia map, and in right picture for study sites).



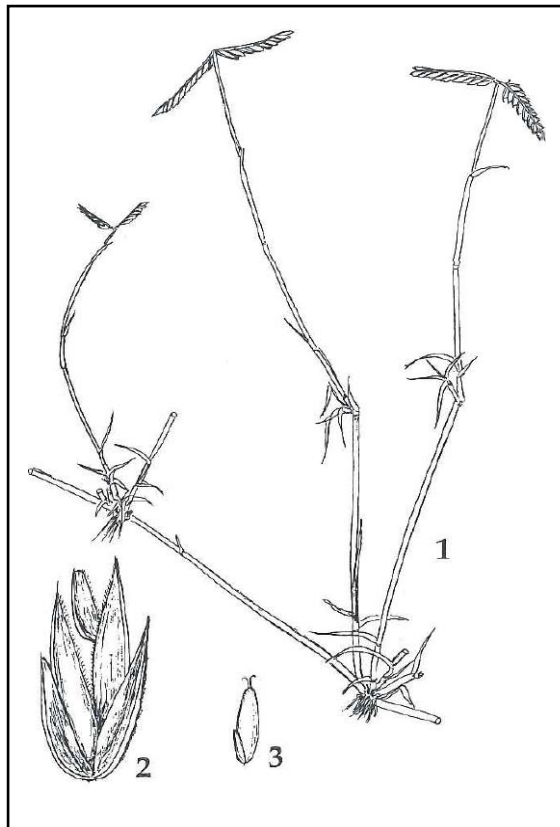
(Figure. 2. The rate monthly rainfall over ten years from 2006 to 2015).



(Figure. 3. The rate monthly temperature over ten years from 2006 to 2015).

### *Plant description*

*Ochthochloa compressa* is a perennial grass that can grow up to 70 cm (Collenette, 1999), and has numerous decumbent stolons that can extend up to 150 cm length, 2-4 mm thick, and produce 1- 6 ramets (personal observation). The blade of the leaf is up to 3-5 cm long, 3 mm wide, and glaucous, the lower leaves are longer than upper leaves. It has two mechanisms of reproduction: sexually by producing seeds in spikes and asexually by producing stolons. The inflorescence contains 3–5 digitate racemes, is 2–4 cm long, and is deciduous upon maturity (Fig. 4). It produces seeds in February and March. *O. compressa* is widespread in the central, northern, and western regions of Saudi Arabia (Collenette, 1999; Mandavilla, 1990), the western and dry tropical regions of Asia, and the northwest dry tropical regions of India and Africa.



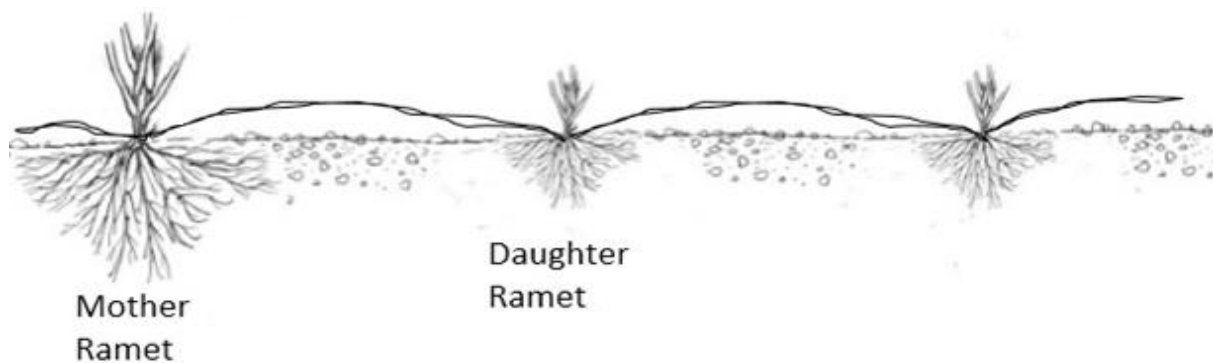
(Figure. 4. The left picture for the morphological shape of *Ochthochloa compressa* (Collenette, 1999), right top picture for *O. compressa* spikes and the bottom left picture for mother ramets.)

### Data collection

I conducted three treatments with nine randomly selected replicates in this study.

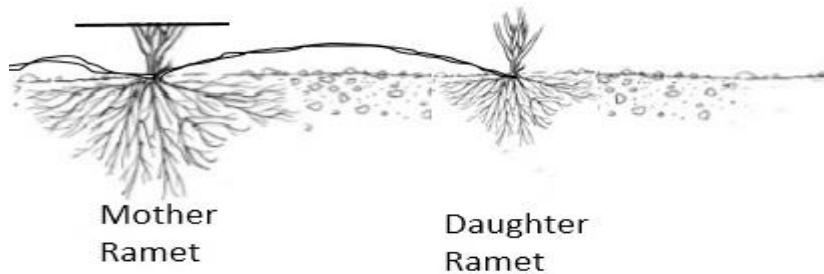
Three treatments and two types of cutting were implemented:

1. The control treatment, which was not exposing to clipping (Fig. 5).



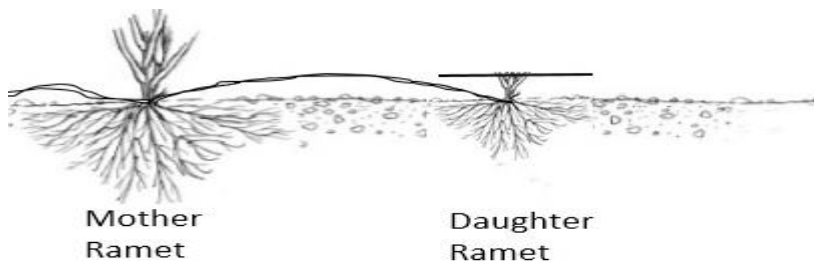
(Figure. 5. Control treatment)

2. The mother ramets grazing treatment, which consisted of cutting and removing 50 – 60% of the mother ramets leaf biomass while they were connected to the daughter ramets (Fig. 6).



(Figure. 6. Mother ramets cutting treatment)

3. The daughter ramets grazing treatment, which consisted of cutting and removing 50 – 60% of the daughter ramets leaf biomass while they were connected to the mother ramets (Fig. 7).



(Figure. 7. Daughter ramets cutting treatment)

I investigated the effect of grazing on the mother and the daughter ramets N, P, and K concentrations and photosynthesis efficiency ( $F_v/F_m$ ). Our experiments, which occurred from the beginning on first week February 2014 until the last week in March 2014.

The concentration of N, P and K were measured and analysed at Ministry of Environment Water & Agriculture in Saudi Arabia by the Kjeldahl method and a spectrophotometer was used to estimate the totals for N (Bremner and Mulvaney, 1982), P and K (Olsem

and Sommers, 1982). For the photosynthesis results, I used an OS30p device (Fig. 8) to measure  $F_v/F_m$  (maximum quantum efficiency) by putting the OS30p device clips with the plant leaves for 20 measures to take a device reading. (Fig. 9).



(Figure. 8. OS30p device (OS-30p+ / Worldwide Trade Thai n.d.).



(Figure. 9. The OS30p device clips on leaf of plants).

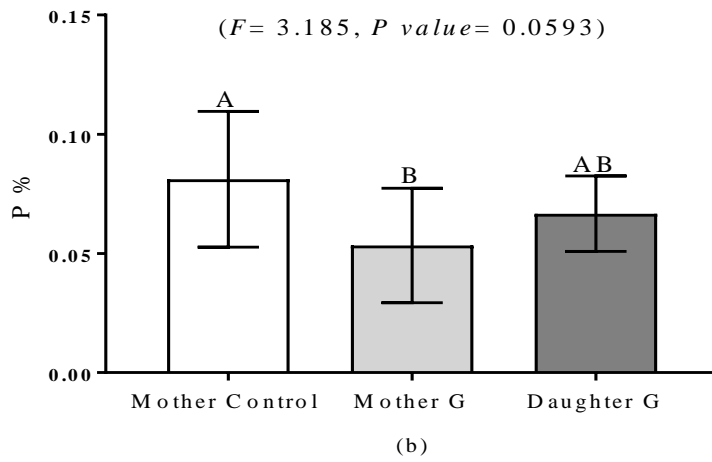
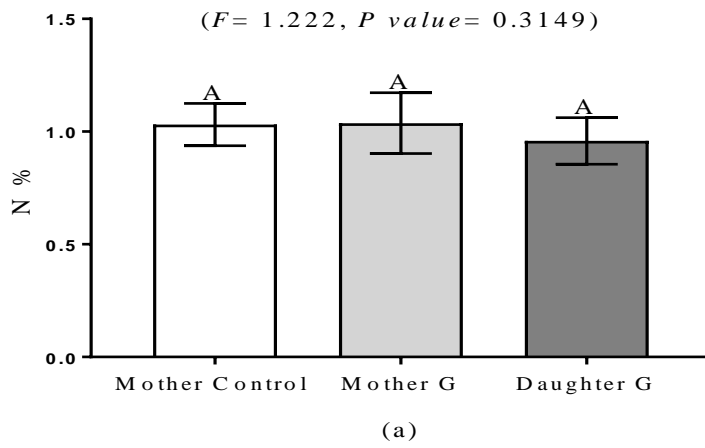
### *Statistical analysis*

The data diagnosis showed that our data had a normal distribution with homogenous variances. One-way ANOVA tests were conducted for the concentrations of N, P and K, then I ran the tests to look for the difference in mean for the mother and daughter ramets, which was performed on all plant samples for an analysis of the total N, P and K in the plant samples. Similar analyses were performed for the effect of grazing on photosynthesis efficiency.

### **Results**

The mother ramets in all three treatments showed no significant difference in total N concentration ( $F = 1.222$ ,  $P = 0.3149$ ) (Fig. 10 a). For the total P concentration, our results showed a marginally significant difference ( $F = 3.185$ ,  $P = 0.0593$ ). The control mother had the highest concentration of total P, while the mother ramets that were clipped had the lowest total P concentration (Fig. 10 b). On the other hand, I found a significant difference in the total K between the mother ramets ( $P < 0.05$ ,  $F = 10.01$ ,  $P = 0.0009$ )

(Fig. 10 c). The mother ramets in the control had the highest concentration of total K, whereas the mother ramets connected to clipped daughters had the lowest concentration of total K.



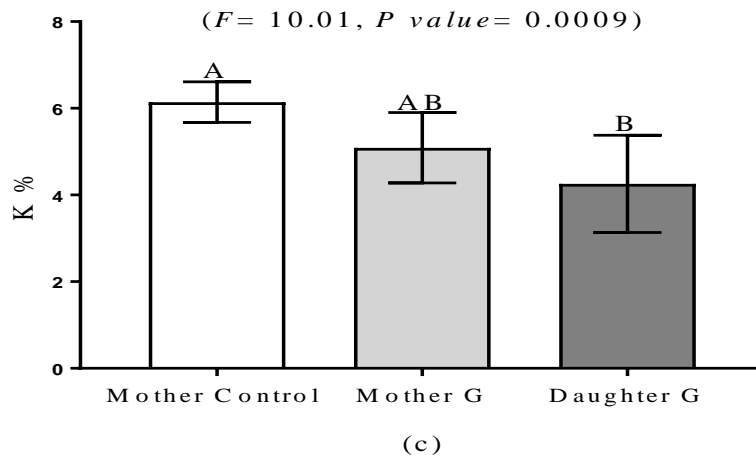


Figure. 10. Concentration of nitrogen (a), phosphorous (b) and potassium (c) in mother ramets in three different treatments: control (Mother control), clipped mother ramets connected to unclipped daughter ramets (Mother G) and unclipped mother ramets connected to clipped daughter ramets (Daughter G).

When I analysed the total N concentration in the samples of the daughter ramets, I found a significant difference in the total N concentration ( $P < 0.05$ ,  $F = 10.01$ ,  $P = 0.0009$ ), although the total N concentration was higher for the daughter ramets when the mother ramets were exposed to grazing (Fig. 11 a).

In the analysis of the total P concentration, I found no significant difference between the daughter ramets samples ( $F = 3.183$ ,  $P = 0.62$ ). The daughter ramets samples recorded the lowest levels of P concentration when the daughter ramets were exposed to grazing, unlike the other daughter ramets samples (Fig. 11 b).

In the analysis of the total K, the daughter ramets samples showed no significant difference between the daughter samples as a result of our experiments ( $F = 1.854$ ,  $P = 0.1814$ ). When I reviewed the results, no significant difference was seen between the daughter ramets' concentration levels in our samples (Fig. 11 c).

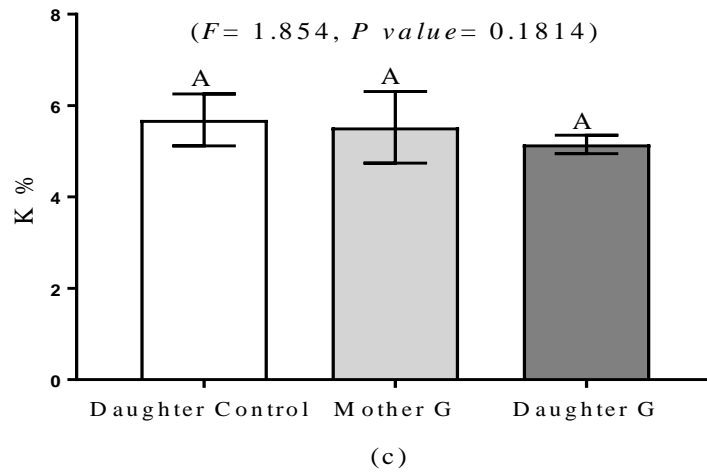
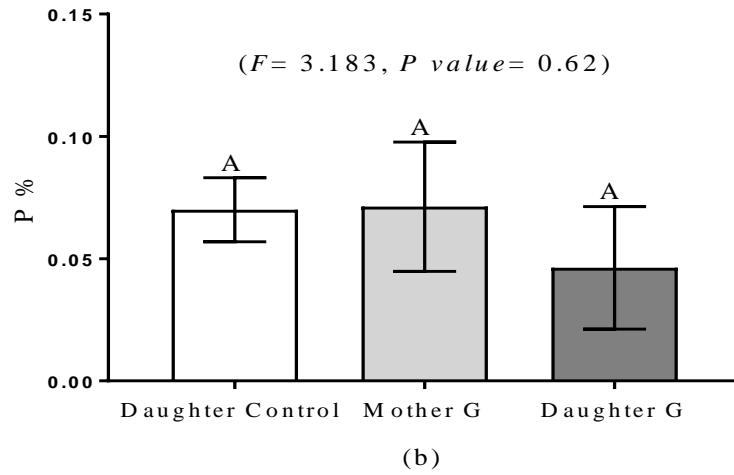
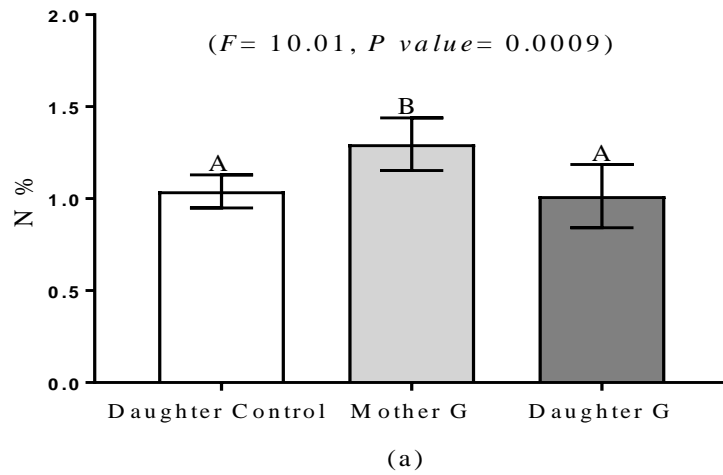
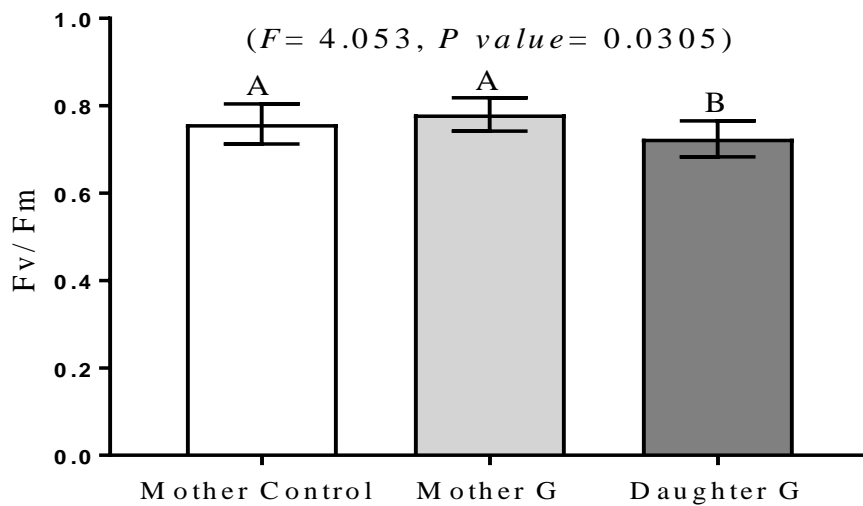
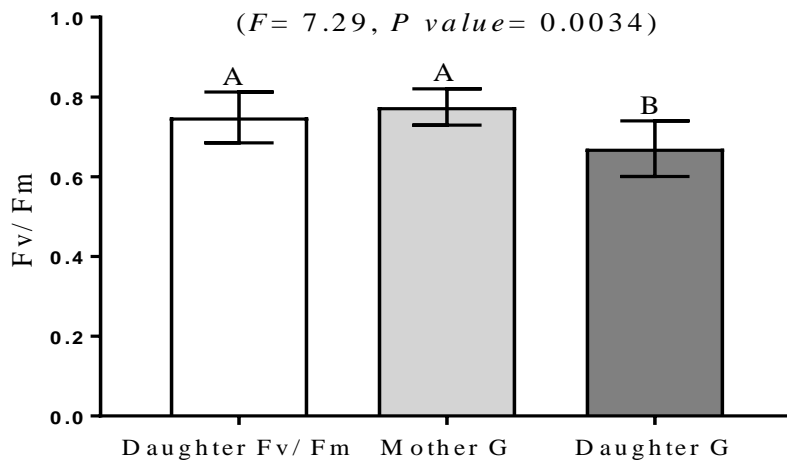


Figure. 11. Concentration of nitrogen (a), phosphorous (b) and potassium (c) in daughter ramets in three different treatments: control experiment (Daughter control), unclipped daughter ramets connected to clipped mother ramets (Mother G) and clipped daughter ramets connected to unclipped mother ramets (Daughter G).





(a)



(b)

Figure. 12. The efficiency of photosynthesis for mother ramets (a) and daughter ramets (b) in three different treatments which is control treatment (Mother control and Daughter control), exposed mother ramets to clipping while connected daughter ramets (Mother G) and exposed daughter ramets to clipping while connected mother ramets (Daughter G).

The analysis of photosynthetic efficiency showed a significant difference when the mother ramets were exposed to clipping ( $P < 0.05$ ,  $F = 4.053$ ,  $P = 0.0305$ ) (Fig. 12 a).

Correspondingly, the daughter ramets samples recorded a significant difference that was more than the mother ramets samples ( $P < 0.05$ ,  $F = 7.29$ ,  $P = 0.0034$ ) (Fig. 12 b).

## Discussion

I found that there was no significant difference in the concentration of nitrogen for mother ramets, when exposing mother or daughter ramets to a defoliation or clipping (Fig. 10 a), probably because the lost energy was compensated by the energy stored either in the roots (Liu *et al.*, 2007; Suzuki and Stuefer, 1999), or stolons (Suzuki and Stueferon, 1999). To be more specific, the apex removal seemingly led to stimulate the transfer of the stored energy in the roots to the above ground tissue which was damaged by clipping. Compensation of lost energy can be obtained through increasing both the activity of young buds which survived clipping and increasing the activity of the meristematic cells (Zhao *et al.*, 2008; Ferraro and Oesterheld, 2002). This was supported by Zhao *et al.* (2008) in their study. They found that exposing mother ramets to moderate grazing (i.e. removal of by 50%) contributed to an increase in the nitrogen concentration compared to other treatments.

With respect to phosphorus and potassium, significant differences were found when exposing mother ramets or connected daughter ramets to clipping compared the control experiments (Fig. 10 b, c). That is to say, a decrease in phosphorus and potassium concentration was recorded which might be due to scarcity of water in soil (Zhao *et al.*, 2008), implying that the plant roots need to be sufficient in soil moisture content with a view to absorbing the phosphorus and potassium. However, this limits growth and weakens the mechanism of compensation of lost tissue (Chapin and McNaught, 1989). Furthermore, persistence of *O. compressa* when compensating the loss of tissue and growth with a lack of phosphorus and potassium concentration and the increased concentration of nitrogen referred that the *O. compressa* does not require phosphorus and potassium to grow in this region (Zhao *et al.*, 2008).

In terms of the daughter ramet treatment, the nitrogen concentration showed significant differences, compared to other treatments when the mother ramets were exposed to clipping. This (i.e. exposing mother ramets to clipping) also led to stimulation and an increase in the activity of young buds to enhance exporting nutrients from mother ramets or stolons to daughter ramets, with a view to compensating the loss in the above ground tissue. Also, clonal plants need nitrogen to enhance sharing sources and nutrients between each other (Alpert, 1996), where the nitrogen, obtained by the daughter ramets from the mother ramets, was more than the nitrogen obtained by the soil (Alpert, 1996), indicating that the concentration of nitrogen in the daughter ramets (personal observation) was not affected by the total size of the roots. The continued connection between mother and daughter ramets, as well as survival of roots and stolons from harm played a role in resistance of grazing and stability of nitrogen concentration in the daughter ramets.

The continued support of mother ramets to the daughter ramets must be obtained through exporting of both water, nutrients and energy to the affected parts of stolons, which often serve as the storages of energy for clonal plants, to use them when required (Kemball and Marshall, 1995; Zhang *et al.*, 2002; Alpert, 1996). The daughters' ramets continue to connect with mother ramets producing sum roots to depend on themselves and be separated from the mother ramets at a later time (Kemball and Marshall, 1995).

The daughter ramets did not record significant differences, unlike daughter connected to mother which recorded the highest concentration among other treatments. This might be due to the increase in the clipped mother's activity in the export of nutrients to other daughters. However, the daughter ramets that were exposed to clipping recorded lower concentrations than the mother, although the mother ramets were exposed to clipping and were not affected (Zhao *et al.*, 2008; Chapin and McNuaghton, 1989). The scarcity of the water, rain and lowering soil moisture might have made it difficult the absorption

phosphorus and potassium by roots to export it towards the upper tissues through xylem (Headley et al., 1988). In addition the daughter ramets have tiny little amount of roots, which resulted in weakness in absorption of phosphorus and potassium from roots area.

There is a consensus that, photosynthesis mainly relies on the above ground tissue or stored energy (Wang *et al.*, 2004), where the photosynthesis are exported horizontally from the mother ramets in the direction of daughter ramets. In this respect, in an attempt to understand the effect of clipping or grazing on both mother and daughter ramets, I measured the photosynthetic efficiency for mother and daughter ramets at all *O. compressa* treatments.

The effect of clipping on the efficiency of photosynthesis showed no significant differences when the mother ramets were exposed to clipping. The most likely reason behind this was that the removal of the topical portions caused an increase in both the activity of the meristematic cells (Zhao *et al.*, 2008) and the stimulation of the lateral buds that survived from clipping to growth, and accordingly performance of photosynthesis became better than apical buds (Zhao *et al.*, 2008). On the other hand, the daughter ramets which were exposed to clipping showed significant differences because of the absence of young buds that sufficiently contributed to both production of photosynthesis instead of the tissue lost and reduction in the leaf area (Zhang *et al.*, 2002). It was found in a previous experiment that cutting the stolons between mother and daughter in *O. compressa* species caused death of the whole stolons and ramets on it, owing to the stop of fluxes of the sources (water, carbohydrate, nutrient, etc.) from mother to daughter ramets (personal observation).

I believe that the result of this experiment provides an insight into understanding the relationship between mother ramets and daughter ramets in *O. compressa* through the

analysis of the concentration of nitrogen, phosphorus and potassium and measuring the efficiency of photosynthesis under the influence of moderate clipping on both mother and daughter ramets while connected. Accordingly, generally speaking, our results showed that in the mother ramets, the concentration of nitrogen was increased and the concentration of phosphorus was decreased. As regards the efficiency of photosynthesis, it was not affected in the mother ramets, however it was decreased in the daughter ramets.

## References

- Al Musallam, MS** 2007, 'Effect of Protection on Vegetation and Soil Seed Bank in Thumama Area' MA thesis, King Saud University, Riyadh.
- Alpert, P & Mooney, H** 1986. 'Resource sharing among ramets in the clonal herb, *Fragaria chiloensis*', *Oecologia*, vol. 70, no. 2, pp. 227-233.
- Alpert, P** 1991. 'Nitrogen sharing among ramets increases clonal growth in *Fragaria chiloensis*', *Ecology*, vol. 72, no. 1, pp. 69-80.
- Alpert, P** 1996. 'Nutrient sharing in natural clonal fragments of *Fragaria chiloensis*', *Journal of Ecology*, vol. 84, pp. 395-406.
- Amsberry, L., Baker, M.A., Ewanchuk, P.J., Bertness, M.D** 2000. 'Clonal integration and the expansion of *Phragmites australis*. *Ecological applications*, vol. 10, no. 4, pp. 1110-1118.
- Bach, CE** 2000. 'Effects of clonal integration on response to sand burial and defoliation by the dune plant *Ipomoea pes-caprae* (Convolvulaceae)', *Australian Journal of Botany*, vol. 48, no. 2, pp. 159-166.
- Belsky, A** 1986. 'Does herbivory benefit plants? A review of the evidence', *The American Naturalist*, vol. 127, no. 6, pp. 870-892.
- Bremner, J.M., Mulvaney, C.S** 1982. Methods of soil analysis, part 2 chemical and Micro biological properties, 595-624.

- Brown, J.F** 1997. 'Effects of experimental burial on survival, growth, and resource allocation of three species of dune plants'. *Journal of Ecology*, pp. 151-158.
- Chapin III, FS, Autumn, K & Pugnaire, F** 1993. 'Evolution of suites of traits in response to environmental stress', *The American Naturalist*, vol. 142, pp. S78-S92.
- Chapin, FS & McNaughton, S** 1989. 'Lack of compensatory growth under phosphorus deficiency in grazing-adapted grasses from the Serengeti Plains', *Oecologia*, vol. 79, no. 4, pp. 551-557.
- Collenette, S** 1999. 'Wildflowers of Saudi Arabia', National Commission for Wildlife Conservation and Development (NCWCD), Riyadh.
- D'Hertefeldt, T & Falkengren-Grerup, U** 2002. 'Extensive physiological integration in *Carex arenaria* and *Carex disticha* in relation to potassium and water availability'. *New Phytologist*, vol. 156, no. 3, pp. 469-477.
- de Kroon, H & Knops, J** 1990. 'Habitat exploration through morphological plasticity in two chalk grassland perennials', *Oikos*, pp. 39-49.
- de Kroons, H & Hutchings, MJ** 1995, 'Morphological plasticity in clonal plants: the foraging concept reconsidered', *Journal of Ecology*, pp. 143-152.
- Dong, M, During, HJ & Werger, MJ** 1997. 'Clonal plasticity in response to nutrient availability in the pseudoannual herb, *Trientalis europaea* L', *Plant Ecology*, vol. 131, no. 2, pp. 233-239.
- Evans, JP & Cain, ML** 1995, 'A spatially explicit test of foraging behavior in a clonal plant', *Ecology*, pp. 1147-1155.
- Ferraro, DO & Oesterheld, M** 2002. 'Effect of defoliation on grass growth. A quantitative review', *Oikos*, vol. 98, no. 1, pp. 125-133.

- He, WM, Alpert, P, Yu, FH, Zhang, LL & Dong, M** 2011. 'Reciprocal and coincident patchiness of multiple resources differentially affect benefits of clonal integration in two perennial plants', *Journal of Ecology*, vol. 99, no. 5, pp. 1202-1210.
- Headley, A, Callaghan, T & Lee, J** 1988. 'Phosphate and nitrate movement in the clonal plants *Lycopodium annotinum* L. and *Diphasiastrum complanatum* (L.) Holub', *New Phytologist*, vol. 110, no. 4, pp. 487-495.
- Kemball, W & Marshall, C** 1995. 'Clonal integration between parent and branch stolons in white clover: a developmental study', *New Phytologist*, vol. 129, no. 3, pp. 513-521.
- Liu, H-D, Yu, F-H, He, W-M, Chu, Y & Dong, M** 2007. 'Are clonal plants more tolerant to grazing than co-occurring non-clonal plants in inland dunes?', *Ecological research*, vol. 22, no. 3, pp. 502-506.
- Liu, H-D, Yu, F-H, He, W-M, Chu, Y & Dong, M** 2009. 'Clonal integration improves compensatory growth in heavily grazed ramet populations of two inland-dune grasses', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 204, no. 4, pp. 298-305.
- Luo, W & Zhao, W** 2015. 'Effects of wind erosion and sand burial on growth and reproduction of a clonal shrub', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 217, pp. 164-169.
- Mandavilla, J. P** 1990. 'Flora of Eastern Saudi Arabia', Kegan Paul International Limited, London.
- Martina, J & Von Ende, C** 2013. 'Increased spatial dominance in high nitrogen, saturated soil due to clonal architecture plasticity of the invasive wetland plant, *Phalaris arundinacea*', *Plant Ecology*, vol. 214, no. 12, pp. 1443-1453.
- Nowak, R & Caldwell, M** 1984. 'A test of compensatory photosynthesis in the field: implications for herbivory tolerance', *Oecologia*, vol. 61, no. 3, pp. 311-318.

- Oborny B, Mony C, Herben T** 2012. 'From virtual plants to real communities: a review of modelling clonal growth'. *Ecological Modelling*, vol. 234, pp. 3-19.
- Olsem, S.R & Sommers, L.E** 1982. Phosphorus. In: A.L. Page, R.H. Miller (Eds). *Methods of Soil Analysis. Part 2. 2nd ed. Agronomy Monograph 9, ASA and SSSA, Madison, WI, pp. 403-430.*
- OS-30p+ / Worldwide Trade Thai** n.d., Worldwide Trade Thai, viewed 5 August 2017, <<http://www.worldwidetradethai.com/?product=os-30p> >.
- Peltzer, DA** 2002, 'Does clonal integration improve competitive ability? A test using aspen (*Populus tremuloides* [Salicaceae]) invasion into prairie', *American journal of botany*, vol. 89, no. 3, pp. 494-499.
- Pennings, SC & Callaway, RM** 2000, 'The advantages of clonal integration under different ecological conditions: a community-wide test', *Ecology*, vol. 81, no. 3, pp. 709-716.
- Roiloa, SR, Alpert, P, Tharayil, N, Hancock, G & Bhowmik, PC** 2007, 'Greater capacity for division of labour in clones of *Fragaria chiloensis* from patchier habitats', *Journal of Ecology*, vol. 95, no. 3, pp. 397-405.
- Strauss, SY & Agrawal, AA** 1999. 'The ecology and evolution of plant tolerance to herbivory', *Trends in Ecology & Evolution*, vol. 14, no. 5, pp. 179-185.
- Stuefer, JF, During, HJ & de Kroon, H** 1994. 'High benefits of clonal integration in two stoloniferous species, in response to heterogeneous light environments', *Journal of Ecology*, pp. 511-518.
- Stueffer, J, De Kroon, H & During, H** 1996, 'Exploitation of environmental heterogeneity by spatial division of labor in a clonal plant', *Functional Ecology*, pp. 328-334.



- Suzuki, JI & Stuefer, J** 1999. 'On the ecological and evolutionary significance of storage in clonal plants', *Plant Species Biology*, vol. 14, no. 1, pp. 11-17.
- Wang, N, Yu, F-H, Li, P-X, He, W-M, Liu, F-H, Liu, J-M & Dong, M** 2008. 'Clonal integration affects growth, photosynthetic efficiency and biomass allocation, but not the competitive ability, of the alien invasive *Alternanthera philoxeroides* under severe stress', *Annals of Botany*, vol. 101, no. 5, pp. 671-678.
- Wang, Z, Li, L, Han, X & Dong, M** 2004, 'Do rhizome severing and shoot defoliation affect clonal growth of *Leymus chinensis* at ramet population level?', *Acta Oecologica*, vol. 26, no. 3, pp. 255-260.
- Wilsey, B** 2002. 'Clonal plants in a spatially heterogeneous environment: effects of integration on Serengeti grassland response to defoliation and urine-hits from grazing mammals', *Plant Ecology*, vol. 159, no. 1, pp. 15-22.
- Wolfer, SR & Straile, D** 2012. 'To share or not to share: clonal integration in a submerged macrophyte in response to light stress', *Hydrobiologia*, vol. 684, no. 1, pp. 261-269.
- Xiao, Y, Tang, J, Qing, H, Ouyang, Y, Zhao, Y, Zhou, C & An, S** 2010. 'Clonal integration enhances flood tolerance of *Spartina alterniflora* daughter ramets', *Aquatic Botany*, vol. 92, no. 1, pp. 9-13.
- Yu, F, Chen, Y & Dong, M** 2002. 'Clonal integration enhances survival and performance of *Potentilla anserina*, suffering from partial sand burial on Ordos plateau, China', *Evolutionary Ecology*, vol. 15, no. 4-6, pp. 303-318.
- Yu, F, Dong, M & Krüsi, B** 2004. 'Clonal integration helps *Psammodochloa villosa* survive sand burial in an inland dune', *New Phytologist*, vol. 162, no. 3, pp. 697-704.

- Yu, F.H., Wang, N., He, W.M., Chu, Y & Dong, M** 2008. 'Adaptation of rhizome connections in drylands: increasing tolerance of clones to wind erosion', *Annals of Botany*, vol. 102, no. 4, pp. 571-577.
- Zhang, C, Yang, C & Dong, M** 2002. 'Clonal integration and its ecological significance in *Hedysarum laeve*, a rhizomatous shrub in Mu Us Sandland', *Journal of plant research*, vol. 115, no. 2, pp. 0113-0118.
- Zhao, W, Chen, S-P & Lin, G-H** 2008. 'Compensatory growth responses to clipping defoliation in *Leymus chinensis* (*Poaceae*) under nutrient addition and water deficiency conditions', *Plant Ecology*, vol. 196, no. 1, pp. 85-99.

## General Conclusion

### Chapter 6

## General Conclusion

Clonal integration is a form of asexual reproduction (Mendoza and Franco, 1998). Clonal plants usually resort to this mechanism as a way to repopulate in poor environments (Luo and Zhao, 2015). The clonal plant usually replicates itself through the production of rhizomes and stolons; however, whether it establishes a new ramet depends on its ability to become separated from the mother ramet and thus become independent and identical to the mother ramets genetically (Wolfer and Sraile, 2012; Yan *et al.*, 2013). Clonal plants use the clonal integration strategy to extend and spread in heterogeneous environments (De Kroon and Hutchings, 1995; De Kroon *et al.*, 2009) and into open spaces (Wang *et al.*, 2008). This enables the clonal plants to share water, carbohydrates and nutrients among ramets (Peltzer, 2002; Xiao *et al.*, 2011). To comprehend the clonal integration mechanism's resistance to variable environmental conditions and to understand the spreading and extension mechanism of *Ochthochloa compressa*, I focused on analysing nitrogen, phosphorus and potassium contents in both mother and daughter ramets; I also measured the effect that competition, grazing and additional nutrients for mother and daughter ramets had on biomass and photosynthesis efficiency. The daughter ramets were studied when connected to and separated from the mother ramets.

One part of the study focused on the extent of the benefits of the mother ramets from daughter ramets when nutrients were added to both groups as the daughter ramets were in contact with or separated from the mother ramets. The results showed that daughter ramets obtain resources from mother ramets. When adding the nutrients to mother ramets, their content of nitrogen, phosphorus or potassium did not change; however, the content of nutrient in daughter ramets increased showing unidirectional flow of nutrients. This

suggests that mother invert strongly in supporting other ramets. While the addition of nutrients to the daughter ramets led to a significant difference in the daughter ramets' retention of nutrients, it did not extend to the passing of nutrients to surrounding ramets or to mother ramets, thus causing to an increase in resources available for the daughter ramets. Furthermore, cutting the stolons between mother and daughter ramets caused death of all ramets on the separated stolons, no significant differences were recorded in terms of the photosynthesis efficiency of either mother or daughter ramets.

To determine whether the stolons spread and the ramets became established by chance, or are directed to richer patches soil samples were collected from six sites: underneath the mother ramets, underneath the first- and second-daughter ramets, the area free of vegetation surrounding the mother ramets at a distance of 50 cm (free area), and the empty patches (naked areas) surrounding the patch where the mother and daughter ramets grew. The analysis indicated significant differences between the samples from the patches surrounding the mother ramets and those from the naked area; the former, for example, had more nitrogen. Regarding phosphorus, the naked area sample displayed higher levels than the other areas' samples. In contrast, the potassium analysis results showed significant differences, with the highest levels recorded in the naked area and free area (at 50 cm distance). This analysis showed us that the mother ramets randomly distributed the stolons and ramets and did not target patches with higher nutrient availability.

When investigating the ability of mother ramets to supply daughter ramets with nutrients and expansion the stolons under harsh environmental condition, it is important to look at the concentration of nitrogen, phosphorous and potassium for both mother and daughter ramets. The analysis indicated no significant differences in the concentrations of nitrogen and phosphorus between mother and daughter ramets. In contrast, the potassium analysis showed significant differences between mother and daughter ramets. The efficiency of

photosynthesis measurements did not indicate any significant differences between the mother and daughter samples, which suggests that an ability of mother to supply a new ramets with nutrients and resistance of the harsh environmental condition and competition.

I also studied the effects of simulated grazing on the clonal plants and the role of clonal integration in grazing resistance. The analysis showed that there was a difference in the results of the mother and daughter ramets. When both mother and daughter ramets were exposed to simulated grazing, they showed no significant differences in nitrogen but did show significant differences in phosphorus and potassium. Daughter and mother ramets showed significant differences for nitrogen when only the mother ramets were exposed to grazing, though there were no significant differences recorded in phosphorus or potassium. In addition, the efficiency of photosynthesis measurements did not indicate significant differences between the mother and daughter ramets when they were exposed to grazing. This demonstrate the importance of clonal integration in *O. compressa* with regard to resistance to grazing through the mechanism of clonal integration. Moderate clipping with sufficient light as in our study might be helpful, through breaking the apical dominance of leaves and buds and motivating the grazed plants to produce more ramets (Zhao et al., 2008). A number of plants might be using tillers and lateral movement of resources through lateral tissue (Price *et al.*, 1992), in order to both compensate nutrient and absent energy and continue to support other ramets (Price *et al.*, 1992).

To contribute to the understanding of the clonal integration mechanism and the understanding of clonal plant behaviour, I seek to conduct in the future further longer-term experiments for at least 12 months.

To understand the age when daughter ramets begin to depend on themselves for nutrients and how they eventually begin acting as independent mother ramets, I need to conduct a long-term experiment in a greenhouse with an environment that approaches the natural conditions. In addition, the daughter ramets need to be separated from the mother ramets at certain intervals. I can then determine the beginning of the daughter ramets' dependence on themselves through the survival of the stolons and the continuation of the daughter ramets' growth. This has clear implications for the management and propagation of this plant with high potential as source of fodder and as a restoration agent for degraded rangelands.

The period from June to October comprises the driest summer season, during which high temperatures are often recorded. These conditions are the most difficult for plants in arid areas. In a future study, I will aim to understand the behaviour of clonal plants (*O. compressa*) when I give them water in such an environment. Does *O. compressa* still expand and spread its stolons, or will it produce seeds? How the plant balances between the opportunities of sexual and asexual reproduction is a critical aspect of its life history strategy. I will also analyse the concentration of nitrogen, phosphorus and potassium in *O. compressa* and compare it with the results to those for the samples without added water.

Due to the lack of time, imposed by the restrictions of a PhD project and the large distance from the field of study and the lack of sufficient financial support during the study period, I will reconduct some experiments so that I can perform a broader and more accurate investigation.

## References

- de Kroon, H, Visser, EJ, Huber, H, Mommer, L & Hutchings, MJ** 2009. 'A modular concept of plant foraging behaviour: the interplay between local responses and systemic control', *Plant, Cell & Environment*, vol. 32, no. 6, pp. 704-712.
- de Kroon, H & Hutchings, MJ** 1995. 'Morphological plasticity in clonal plants: the foraging concept reconsidered', *Journal of Ecology*, pp. 143-152.
- Luo, W & Zhao, W** 2015. 'Effects of wind erosion and sand burial on growth and reproduction of a clonal shrub', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 217, pp. 164-169.
- Mendoza, A & Franco, M** 1998. 'Sexual reproduction and clonal growth in *Reinhardtia gracilis* (Palmae), an understory tropical palm', *American journal of botany*, vol. 85, no. 4, pp. 521-521.
- Peltzer, DA** 2002. 'Does clonal integration improve competitive ability? A test using aspen (*Populus tremuloides* [Salicaceae]) invasion into prairie', *American journal of botany*, vol. 89, no. 3, pp. 494-499.
- Price, EA, Marshall, C & Hutchings, MJ** 1992, 'Studies of growth in the clonal herb *Glechoma hederacea*. I. Patterns of physiological integration', *Journal of Ecology*, pp. 25-38.
- Wang, N, Yu, F-H, Li, P-X, He, W-M, Liu, F-H, Liu, J-M & Dong, M** 2008. 'Clonal integration affects growth, photosynthetic efficiency and biomass allocation, but not the competitive ability, of the alien invasive *Alternanthera philoxeroides* under severe stress', *Annals of Botany*, vol. 101, no. 5, pp. 671-678.
- Wolfer, SR & Straile, D** 2012. 'To share or not to share: clonal integration in a submerged macrophyte in response to light stress', *Hydrobiologia*, vol. 684, no. 1, pp. 261-269.

- Xiao, Y, Tang, J, Qing, H, Zhou, C & An, S** 2011. 'Effects of salinity and clonal integration on growth and sexual reproduction of the invasive grass *Spartina alterniflora*', *Flora-Morphology, Distribution, Functional Ecology of Plants*, vol. 206, no. 8, pp. 736-741.
- Yan, X, Wang, H, Wang, Q & Rudstam, LG** 2013. 'Risk spreading, habitat selection and division of biomass in a submerged clonal plant: Responses to heterogeneous copper pollution', *Environmental pollution*, vol. 174, pp. 114-120.
- Zhao, W, Chen, S-P & Lin, G-H** 2008, 'Compensatory growth responses to clipping defoliation in *Leymus chinensis* (Poaceae) under nutrient addition and water deficiency conditions', *Plant Ecology*, vol. 196, no. 1, pp. 85-99.