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Hans H. Ruthenberg Award for Graduates 2007

Blen Beyene "Drought stress effect on the glucosinolate content of Brassica carinata A. Braun"

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Summary

Traditional leafy vegetables (TLVs) are groups of leafy vegetables that have evolved naturally in a given area or have been introduced and then adapted so well that they have become an integral part of the local food system and diet. TLVs were traditionally gathered from the wild. As their occurrence in the wild became limited and inconvenient, farming communities started to cultivate some of them.

TLVs play an important role in satisfying the "hidden hunger" (deficiency of micronutrients, vitamins and minerals whose impact on the body is so profound relative to the amount needed). Furthermore, TLVs are important in food security during times of drought or poor harvest and are also vital for income generation.

Brassica carinata is one of the major TLVs in east Africa in particular Ethiopia. It is consumed during the rainy season, when it grows in abundance and during drought when grains are in short supply. The edible parts of this plant are the leaves and the seeds. The leaves are stripped off and eaten as a vegetable whereas the seed is pressed as a source of oil. Being a member of the Brassicaceae family, *B. carinata* synthesizes secondary plant metabolites mainly glucosinolates. Glucosinolates are sulfur rich secondary metabolites that commonly occur in the Brassicaceae family. The hydrolysis products of glucosinolates have a deterring effect on herbivore pests and may have anti-nutritional effects in animal feedstuffs. In human nutrition, depending on the type of glucosinolates, they may cause bitterness and pungent taste. The anticarcinogenic effect of glucosinolates has also been reported in different vegetables. Meanwhile, glucosinolates concentrations are highly variable depending on genotype, tissue type, physiological age and growing condition making the anticipation of positive and negative effect in human nutrition difficult.

Therefore, the main objectives of this project were to investigate the effect of drought stress on the concentrations of glucosinolates in the leaf of *B. carinata* as well as examining the differences in drought adaptation mechanisms of two *B. carinata* varieties in relation to glucosinolate concentrations in leaves. The expected result was that under drought stress the

concentrations of glucosinolates in leaves will increase and positively influence drought adaptation mechanisms of plants.

All experiments were conducted on soil filled pots under controlled growing condition in the glasshouse of the Institute of Biological Production Systems, University of Hannover, Germany in the summer of 2005 and 2006. During the first part of the project, four *B. carinata* varieties of Ethiopian source (Holeta-1, 37-A, Yellowdodola and PVT ES 2/13/12) were used to identify the leaf glucosinolate profile. Plants were grown under optimal irrigation till they reached the 10th leaf stage. Whole plant leaves were taken as a sample and analyzed for glucosinolates using HPLC. In all varieties the profile was mainly composed of four glucosinolates, namely Glucobrassicin, Neoglucobrassicin 4-Methoxy glucobrassicin and Sinigrin, which constitutes the largest share. On the other hand, all the test varieties showed significant variations of total leaf glucosinolates concentration, where 37-A showed the highest and Holeta-1 showed the least.

In the second part of the experiment the two varieties of *B. carinata* that showed the most and the least leaf glucosinolates concentration (37-A and Holeta-1, respectively) were used. Two water treatments were implemented using two different irrigation schemes. One group was watered every other day in order to maintain 80% pot capacity. Drought stress was applied to the second group by withholding irrigation in which the pot capacity could drop to 20%. Data were collected on growth parameters, plant water relations, total glucosinolate contents and the taste of leaves. Whole plant leaf samples were gathered four times at 5-6, 7-8, 9-10 and 11-13 leaf stages. Leaf glucosinolate concentration was identified using HPLC and Sinigrin as internal standard.

Drought stress caused a reduction in growth parameters of both varieties. Under drought stress plants of both varieties showed reduced leaf expansion rate and accumulated little dry matter. At the same time, the evapotranspiration rate of both varieties dropped significantly. Their water use efficiency, an indicator to drought avoidance, increased but as the drought stress intensified the water use potential also started to decline. Both varieties also showed physiological age dependent osmotic adjustment, where younger leaves tried to tolerate the drought stress by adjusting their osmotic potential to lower levels. In general reduced relative water content correlated with the decrease in cumulative evapotranspiration, whole plant leaf area and osmotic potential of variety 37-A. Similarly in Holeta-1 reduced relative water content correlated to a decrease in water potential and cumulative evapotranspiration.

Furthermore, drought stress elevated leaf glucosinolates content of both varieties considerably. However, the magnitude of glucosinolate concentration depended on the stage of development and the intensity of the drought stress. Meanwhile, in both varieties the increase in leaf glucosinolate concentrations correlated with relative water content (Fig. 1), where reduced water content led to higher leaf glucosinolate concentration. However, higher leaf glucosinolate concentration did not correlate with dry matter accumulation and osmotic adjustment. Consequently, the contribution of glucosinolates towards drought stress resistance in terms of osmotic adjustment and dry matter accumulation was not significant. Meanwhile, as a result of drought stress the taste of leaves of both varieties appeared to be more bitter and harder, particularly during raw consumption. Cooking reversed the effect by leaching out Sinigrin (higher concentration of Sinigrin has been reported to cause bitterness).

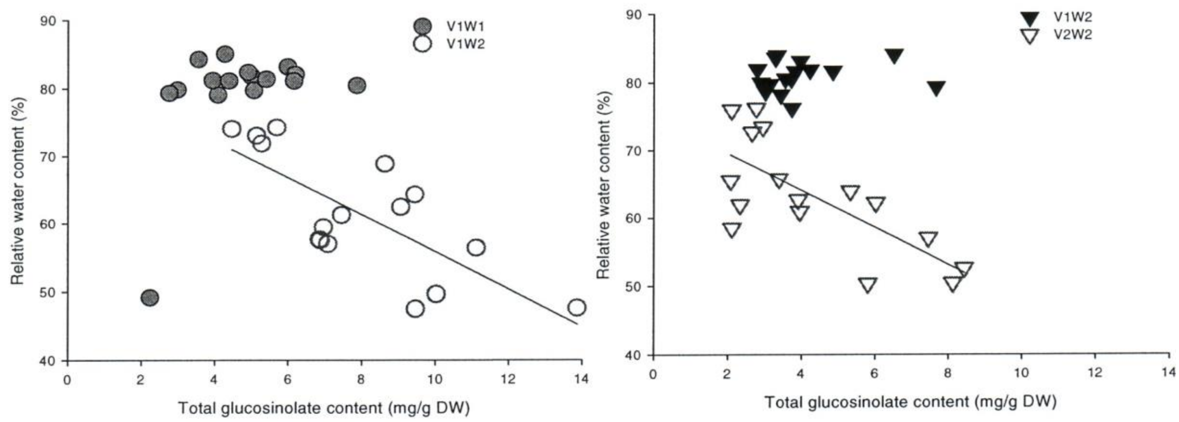

Figure 1. Total glucosinolate contents in relation to Relative water content of the leaves of two B. *carinata* varieties. VI and V2 represent Holeta-1 and 37-A, respectively. Whereas Wl and W2 stands for regularly watered and drought stressed conditions, respectively

Total glucosinolate level of *B. carinata* (36.9±1.13 mg/lOOg FW), was lower than the average concentration in Broccoli, white cabbage and Brussels sprouts. The absence of Progoitrin, whose hydrolysis product Goitrin increases the incidence of goiter by inhibiting the normal functioning of thyroid, in the glucosinolate profile of *B. carinata* makes it safe to be used as TLV. However, the low concentration of Glucobrassicin (the most investigated glucosinolate for its anticarcinogenic activity) reduces the contribution of *B. carinata* to a healthy nutritional diet.

As anticipated in both varieties, leaf glucosinolates contents tended to increase with the drought cycle. The reason behind this effect is not well established yet. However, there are some hypotheses set to explain this phenomenon. The growth-differentiation balance hypothesis (GDBH) that predicts how plants allocate between differentiation related processes and growth-related processes in different environmental conditions. According to GDBH, increased resource availability can either increase or decrease secondary metabolite concentrations, depending on the initial status of the plant. Rapidly growing plants are predicted by the GDBH to have low secondary metabolite concentrations because of a resource-based trade-off between primary and secondary metabolic pathways. However, moderate water or nutrient limitation slows down growth more than carbon assimilation leading to the accumulation of carbohydrates in source leaves that can be used for secondary metabolism. The patterns observed in our results conformed partly what is predicted by GDBH. The concentrations of total glucosinolates were low in the fast-growing, well-watered plants, which is consistent with GDBH. A strong negative correlation was also observed between leaf expansion rate and total glucosinolate concentrations in 37-A. Moreover, increased solute concentration (which might include excess carbohydrate accumulation due to slower growth) in a form of osmotic adjustment is observed in both varieties. The second and third possible explanations are sought from sulfur metabolism and understanding plant hormones and network of signal transduction during drought stress. The alteration of nutrient uptake due to water deficit may lead to an imbalance in sulfur to nitrogen ratio resulting in the accumulation of glucosinolates as sulfur sink. However, this standpoint is debated because of the small share of glucosinolates in the total sulfur content of Brassica crops. Furthermore, stresses such as low water availability change the hormonal distribution of plants leading to a cascade of signal transduction pathways that result in the expression of stress responsive genes. Particularly, stress hormones like abscisic acid (ABA), jasmonic acid (JA), ethylene and salicylic acid (SA) (that play a very important role in biotic and abiotic stress resistance) are known to increase the concentrations of glucosinolates.

The results of our experiments supported the predictions of GDBH. Direct relation between drought and sulfur/nitrogen fertilization needs to be investigated and the effect of application of plant hormones like SA, JA and ABA needs to be proven in this particular species.
In conclusion, drought stress caused a reduction of leaf area and plant biomass, and an increase in glucosinolate contents that lead to a slight increase in the bitterness and hardness of the leaves of the two varieties of *B. carinata*. Consequently, this might have a negative impact on its use as TLV. However, since the concentrations of glucosinolates show variation, the time of harvest and way of consumption may reduce the negative effects. From a nutritional (medical) point of view, even if the concentrations of indole glucosinolates (glucosinolate with higher anticarcinogenic activity) are limited, the consumption of diets containing leaves of *B. carinata* would have beneficial effects on health. As epidemiological data have shown, an intake of as little as 10 g/day of cruciferous vegetables can reduce the risk of a number of cancers significantly.