STRUCTURE-ACTIVITY RELATIONSHIPS (SAR) STUDY OF WHEAT ALLELOCHEMICALS

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We present the methodology for preparation and evaluation of wheat extracts in order to establish the most suitable variety of wheat with high potential allelopathic activity, and the evaluation of phytotoxic activity of wheat allelochemicals, directly isolated or derived from degradation.

SELECTION OF WHEAT CULTIVAL VARIETIES

A key point was the optimization of a general procedure for collecting, storing and sending samples during the four selected development stages of wheat growth. Wheat was produced in two countries (Denmark and Spain) and following two cultivations procedures (conventional and organic) in order to produce comparative samples from both lands that could be measured and quantitatively analyzed for the whole consortium in different labs.

Plant material needed to be immediately frozen after collection. Samples were cleaned up to eliminate soil and stored trying to avoid desiccation. Whenever possible, the plants were collected with shoots and roots. Any damage in roots was maintained at minimum. The samples were kept frozen until extraction and the fresh weight of the sample will be recorded before freshening.

For fresh sample extraction, a plant weight/water volume ratio of 1/3 was used and samples were processed without de-freshening.

For the extraction of dry plants, the frozen material was kept at 50°C in the incubation chamber for 48 h. The samples were wrapped in filter paper to avoid contamination and leaving them opened in both sides to allow air free circulation. The samples were extracted with water when a constant weight was reached. The usual value corresponded with a 60 % of the initial weight. A dried plant weight/water volume ratio of 1/9 was used for extraction.

The plant material for the extraction was placed in a 50x50 cm drilled tray. The rain was simulated through a water-dispersion device that controlled the size and the number of drops. The plants needed to be arranged in a layer as thin as possible to allow the free circulation of the water drops. The water was collected in a funnel and re-circulated or collected for analysis. The extraction was continued through 24 h and the volume of water is calculated on the basis of the weight of the sample and its conditions (dry or fresh).

A pre-filtration step through double cheesecloth was carried out to eliminate impurities of bigger sizes that might occlude the membrane. The samples obtained through this process were vacuum filtered using a 0.22 µm pore diameter membrane. Once the samples have been filtered, they were stored at -20°C in plastic bottles and kept until bioassay.

This procedure was used with 60 samples: six different wheat varieties from Denmark and Spain grown under organic and conventional conditions were collected at four different development stages.

BIOASSAY RESULTS

The bioassays were carried out in 10 mL test tubes. Five ethiolated coleotiles obtained from the apical part of 3-days-old wheat seedlings were placed in 2 mL of phosphate/citrate buffered solution adjusted at pH 5.6. The tubes were kept under constant agitation in a rotator for 24 h. in the darkness at 22 °C. This solution contains the product or extract to be assayed.

The test solutions are prepared from the mother extract through successive dilution to get final concentrations of 1:5, 1:10, 1:20. 1:40, 1:80, and 1:160. Each assay was made on triplicate, including the control.

The wheat coleoptile length was recorded digitally and treated using Photomed®. This is a digital informatics device developed by our group to obtain accurate measurements in the range of 4-10 mm (typical length of a coleoptile). Data were automatically processed to obtain graphics and the statistics. The statistical treatment of the data is made using the Student´s t test.

The assay results have allow us to proposed for allelopathic studies for large studies the varieties Astron and Ritmo as the most active varieties and Stakkado as the lowest bioactive variety as positive control. Additionally, the third stage of development of wheat growth from organic cultivation, has been selected.

BIOASSAY RESULTS OF ALLELOCHEMICALS

We have tested a number of compounds related with the biosynthesis and degradation of DIBOA in order to evaluate their bioactivity with general and phytotoxicity bioassays and to establish structure-activity relationships.

All compounds were assayed with wheat coleoptile as **general bioactivity** test. The concentration range was from 10^{-3}M to 10^{-5}M, and were compared with the effects of the commercial herbicide LOGRAN©. The cluster analysis of bioactivity data shows three separate groups of compounds: APO with inhibitory activity higher than 75% at 1mM (very similar to the herbicide of reference), APH and DIBOA with activity higher than 40% at the same concentration, and the other ones with weak activity (< 25%).

The high activity measured for APH can be explained for the fact of this compound suffers a very fast degradation to APO during bioassay. After 24 hours of assay we observed an approximate APH /APO ratio 1/4 in the solution recovered from growth medium. This quick degradation was not observed in the case of DIBOA which was transformed to BOA and APO in significant proportion only after 72 hours. This slow degradation was also observed for BOA in bioassays under hydroponics conditions.

The bioactivities shown by the other products derived of the biosynthesis of DIBOA (HBOA) or degradation of APH (HPAA and HPMA) were clearly lower than those of DIBOA and BOA. Moreover, AAPO, product derived of degradation of APO, presented also a weak inhibitory activity. These data can be correlated with possible mechanisms for detoxification for these compounds.

Phytotoxic activities have been evaluated using the bioassay protocol developed by our group. The measured parameters were germination, root length and shoot length. Plant species used were the "Standard Target Species" (STS): *Lepidium sativum* L., *Lactuca sativa* L. and *Lycopersicon aesculentum* Will. as dicots., and *Triticum aestivum* L. and *Allium cepa* L. as monocots. As representative weed species of wheat crops were selected *Lolium rigidum* L. and *Avena fatua* L. LOGRAN© was used as internal standard and the dilutions of the compounds assayed are the same than in the general bioactivity test.

The observed activities depended on the evaluated parameter. The results are separately discussed for the three measured parameters in dicotyledonous and monocotyledonous species.

In general, germination was the parameter less affected. However, there was a clear inhibitory effect of APH and APO over almost all tested species. This fact could be explained as above where

there was clear degradation of APH to APO during bioassays. Other interesting point was the lack of activity of DIBOA over germination of monocotyledonous species, specially over selected weeds. This fact clearly showed how this compound must be marked as possible post-emergence phytotoxic compound, specially if we considered the possible degradation of this compound during this residence time at soil.

Selected data for the activity of tested compounds over root length made possible to establish that most of the assayed compounds showed high levels of phytotoxic activity. APO showed a profile of activity more consistent even than the commercial herbicide formulation LOGRAN©. These data were in good agreement with the idea that phytotoxic activity of wheat is due to degradation of DIBOA to APO more than a direct action of this compound or BOA. This is specially remarkable when we considered lowest concentrations (10^{-4}M to 10^{-5}M). Inhibitory action of APH was well correlated with its degradation to APO during bioassays. The fact of lack of inhibitory effects over dicotyledonous species for other compounds related with synthesis of DIBOA and degradation of APH (HBOA, HPAA and HPMA) was specially significant. However, HPMA showed inhibition for monocotyledonous weeds at 10^{-3}M: 50% over rigid ryegrass (*Lolium rigidum* L.) and 25% over wild oat (*Avena fatua* L.).

Phytotoxic bioactivity results for shoot length showed different effects over tested species. Dicotyledonous species were more affected than monocotyledonous ones. This fact is clearly observed for BOA and in lower degree for DIBOA, which maintained certain inhibitory effects over weeds. For APO there was a difference of at least 30 % between two taxonomic groups. These data suggest some special resistance of weeds to these compounds, which will occur in any case when they are moved to aerial part of the plants.

CONCLUSIONS

- HBOA, intermediate of biosynthesis of DIBOA, does not show high phytotoxic activity, which is agree with the idea that 4-hydroxy group of benzoxazinoids is crucial for the biological activity. These data suggest that the cyclic arylhydroxamic acid group to be the group responsible for the activity.
- APH, is rapidly transformed to APO under bioassays conditions. Its phytotoxic activity must be explained considering the appearance of some quantity of APO during assays.
- General and phytotoxic effects of APO in all plants assayed suggested that this compound could be considered as responsible of higher and persistent phytotoxic activity derived from DIBOA and BOA.
- Final compounds obtained of degradation of APH (AAPO, HPAA, and HPMA) do not show high phytotoxic activity. This is consistent with the hypothesis that these ones could be final products of degradation and/or detoxification of DIBOA that inactivate this allelochemical.
- Wheat plants are resistent to phytotoxic activity of DIBOA and its related compounds, specially at medium concentrations. These data suggest that wheat has developed a mechanism that prevents autotoxicity.