BARD Final Scientific Report

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Date of Submission of the report: December 30, 2005

BARD Project Number: IS-3133-99C

Project Title: Inhibition of the Gravitropic Shoot Bending in Stored Cut Flowers Through Control of their Graviperception: Involvement of the Cytoskeleton and Cytosolic Calcium

Investigators

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Keywords *not* appearing in the title and in order of importance. Avoid abbreviations.

Actin, myosin, microtubules, auxin, amyloplasts, ATPases, cytosolic Ca^{2+} , calcium antagonists, snapdragon spikes, oat shoot pulvini, 1,4,5-trisphosphate, endodermis, cortex, phosphorylation, *Aux-IAA* genes, ethylene, brassinolide.

Abbreviations commonly used in the report, in alphabetical order: BDM, 2,3-butanedione-2-monoxime; $[\text{Ca}^{2+}]_{\text{cyt}}$, cytosolic calcium concentration; CD, cytochalasin D, CDTA, *trans*-1,2-cyclohexane dinitro-*N,N,N',N'*-tetraacetic acid; ER, endoplasmic reticulum; IAA, indole 3-acetic acid; IP_3 , inositol 1,4,5-trisphosphate; Lat B, latrunculin B; MTs, microtubules; NPA, N-1-naphthylphthalamic acid; PLC, phospholipase C; PM, plasma membrane.

Budget: IS: \$ 190,000

US: \$ 100,000

Total: \$ 290,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged		2	2	4
Submitted, in review, in preparation	1		3	4
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts	2		7	9
Not refereed (proceedings, reports, etc.)	2		1	3

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1		1	2
Longer Visits (Sabbaticals)				

Description Cooperation:

Both synergistic and complementary modes of cooperation were employed: Each research group has contributed its specific expertise for studying several aspects or factors of graviperception in snapdragon cut flowers and in oat shoot pulvini as a model system. In this manner we could confirm a more general sequence of events occurring during graviperception and leading to shoot bending.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

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A.2. Abstract

Original objectives: The basic goal of the present project was to study the mechanism involved in shoot graviperception and early transduction, in order to determine the sequence of events operating in this process. This will enable to control the entire process of gravity-induced differential growth without affecting vertical growth processes essential for development. Thus, several new postulated interactions, operating at the perception and early transduction stages of the signaling cascade leading to auxin-mediated bending, were proposed to be examined in snapdragon spikes and oat shoot pulvini, according to the following research goals: **1)** Establish the role of amyloplasts as gravireceptors in shoots; **2)** Investigate gravity-induced changes in the integrity of shoot actin cytoskeleton (CK); **3)** Study the cellular interactions among actin CK, statoliths and cell membranes (endoplasmic reticulum - ER, plasma membrane - PM) during shoot graviperception; **4)** Examine mediation of graviperception by modulations of cytosolic calcium - $[Ca^{2+}]_{cyt}$, and other second messengers (protein phosphorylation, inositol 1,4,5-trisphosphate - IP_3). **Revisions:** **1)** Model system: in addition to snapdragon (*Antirrhinum majus* L.) spikes and oat (*Avena sativa*) shoot pulvini, the model system of maize (*Zea mays*) primary roots was targeted to confirm a more general mechanism for graviperception. **2)** Research topic: brassinolide, which were not included in the original plan, were examined for their regulatory role in gravity perception and signal transduction in roots, in relation to auxin and ethylene. **Background to the topic:** The negative gravitropic response of shoots is a complex multi-step process that requires the participation of various cellular components acting in succession or in parallel. Most of the long-lasting studies regarding the link between graviperception and cellular components were focused mainly on roots, and there are relatively few reports on shoot graviperception. Our previous project has successfully characterized several key events occurring during shoot bending of cut flowers and oat pulvini, including amyloplast displacement, hormonal interactions and differential growth analysis. Based on this evidence, the present project has focused on studying the initial graviperception process in flowering stems and cereal shoots. **Major conclusions and achievements:** **1)** The actin and not the microtubule (MT) CK is involved in the graviperception of snapdragon shoots. **2)** Gravisensing, exhibited by amyloplast displacement, and early transduction events (auxin redistribution) in the gravitropic response of snapdragon spikes are mediated by the acto-myosin complex. **3)** MTs are involved in stem directional growth, which occurs during gravitropism of cut snapdragon spikes, but they are not necessary for the gravity-induced differential growth. **4)** The role of amyloplasts as gravisensors in the shoot endodermis was demonstrated for both plant systems. **5)** A gravity-induced increase in IP_3 level of the upper flank, stabilized later on as a differential distribution of IP_3 in favor of the lower flank, were found both in snapdragon spikes and oat shoot pulvini. **6)** Gravity-induced early differential phosphorylation of two soluble proteins was found in oat shoot pulvini. **7)** Based on studies with various specific inhibitors, we have established the role and involvement of the three second messengers, $[Ca^{2+}]_{cyt}$, IP_3 and protein phosphorylation, in gravitperception leading to auxin redistribution. **8)** The role of $[Ca^{2+}]_{cyt}$ in mediating gravisensing of snapdragon spikes was further established by showing the effects of $LaCl_3$ in modulating the activity of a putative PM calcium channel, and by abolishing the gravity-induced enhancement and activity gradient of the PM and ER calcium pumps. **9)** A possible sequence of events in the gravity signaling chain of shoots was suggested: amyloplast displacement, mediated by the actomyosin complex, induces lateral asymmetric changes in IP_3 , which induce the differential distribution of auxin. This auxin gradient induces in turn differential expression of *Aux/IAA* genes and changes in MT orientation of the upper stem flank, leading to differential stem growth. **Implications - scientific and agricultural:** The innovative results of this project have provided some new insight in the basic understanding of graviperception in shoots that partially filled the gap in our knowledge, and established useful means for its control. Gravitropism has an important impact on agriculture, particularly for controlling the bending of various important agricultural products with economic value. So far, no safe control of the undesired bending problem of flower stalks has been established. The essential characterization of these key events of graviperception and their sequence will lead to development of means to block gravity perception, which eventually will inhibit stem bending without hampering shoot and flower growth after harvest.

A.3. Achievements

A.3.1. Significance of main scientific achievements or innovations

The gravitropic bending response of cut flowers with actively growing spikes after harvest, occurs upon their horizontal placement during shipment, and exhibits a major horticultural problem. Despite long-lasting studies in various graviresponsive systems mainly in roots, and although extensively studied in various aboveground organs, the graviperception was hardly investigated in flowering shoots. Our present project has provided some new insight into the basic understanding and sequence of events operating in the gravity-signaling cascade of flower and cereal shoots, thereby partially filling this gap in knowledge. The significance of the main achievements gained in this project is briefly outlined as follows:

Although the effects of several modulators of gravistimulation have been previously investigated in various organs, little effort was made to determine the timing and interaction with additional events during graviperception. Our research describes a unique attempt to basically understand graviperception events in shoots of cut flowers and cereal grass, by introducing several novel ideas which show considerable promise. Our results show for the first time in shoots, the complex interaction between the gravisensors (amyloplasts supported by the acto-myosin system) and the subsequent transduction events mediated by auxin and additional plant growth regulators (ethylene, brassinolide). We have clearly demonstrated the involvement of three important second messengers, $[Ca^{2+}]_{cyt}$, IP_3 and protein phosphorylation, in these processes. Our findings also supply new information on the involvement of $[Ca^{2+}]_{cyt}$ and actin-CK elements in the gravitropic response of snapdragon shoots. We have established possible hormonal interactions during the gravitropic response, between ethylene and auxin in snapdragon stems, and between ethylene and brassinolide in maize roots. In addition, employing the inhibitor-based approach as a research tool, may possibly lead to a practical benefit to the flower industry, which is of great economic value to both countries.

Based on the findings of this research obtained with snapdragon spikes and confirmed in the model system of oat shoot pulvini, we could establish for the first time a possible sequence of events in graviperception of shoots, as follows: Gravity-dependent disturbance of the actin CK, which do not lead to partial depolymerization of actin filaments, are involved in sedimentation of amyloplasts to a new specific site, possibly by providing a network on which the amyloplasts can migrate with the assistance of the myosin protein. Actin CK and amyloplast interactions with cell membranes (ER, PM) may elicit transient changes in $[Ca^{2+}]_{cyt}$, possibly mediated through IP_3 action. These rapid gravity-induced changes in the various second messengers are subsequently translated into differential distribution of ER Ca^{2+} -ATPase pumps, IP_3 and phosphorylated proteins across the stem, which may serve as signal amplification devices. Such asymmetric messenger mediation can activate membranous auxin receptors, thereby leading to lateral auxin transport in favor of the lower stem

flank and to the corresponding differential expression of early auxin-responsive genes. Consequently, the auxin depletion from the upper stem flank will induce changes in the MT orientation from transverse to oblique and longitudinal arrays. This MT orientation, associated with growth cessation of the upper flank, will result in differential stem growth leading to bending. In this way the tissue can establish an initial asymmetry in graviperception and in the transduction of the signal, for eliciting a differential growth response. Hence, we could demonstrate that since amyloplasts and their specific interaction with the acto-myosin network exist only in shoot endodermis cells, only these cells which have the ability of gravity transduction sensing, will further amplify the signal and communicate it outside through differential expression of various internal factors.

Our results extend the participation of the acto-myosin network in gravitropism to the shoot. This further contributes to the general emerging picture obtained so far in other graviresponding systems that the acto-myosin system is necessary for amyloplast movement during gravistimulation and for the subsequent transduction events leading to bending. Additionally, our results clearly support the Cholodny-Went theory postulating that graviperception leads to auxin redistribution towards the lower side of the gravireacting organ.

Taken together, this study of gravitropism with its rather innovative and unique findings, has shed more light on the process of shoot graviperception and on the complex growth behavior of shoots exposed to changes in the gravity vector. As such, this study suggests possible useful means for controlling these processes, and has therefore important implications for science and agriculture.

A.2.3. Agricultural and/or economic impacts of the research findings

Gravitropism has an important impact on agriculture. It allows plants to compete for the limited resources available in their immediate environment and ensures that crop shoots resume upward growth after lodging. Thus, the anticipated contribution of this project is to fundamental science, horticulture and agriculture, as well as to behavior of plants in space, under weightlessness conditions. Accordingly, elucidation of the mechanisms involved in shoot graviperception may provide better tools for postharvest handling of cut flowers and other stem bending commodities such as asparagus, as well as developing strategies to improve cereal shoot bending after lodging. Thus, the economic benefit and impact of this project is mainly expressed in improvement of all these commercial commodities in terms of increased production and export for cut flowers, or increased yield and minimized losses for cereals and other shoots. This outcome is of great economic value to both countries.

A.3.3. Details of cooperation

The fruitful collaboration set up, established during the previous BARD project between the two research teams, continued also during the present project. This project involves studies in plant physiology, cellular and molecular biology and ornamental horticulture, and represents a combined

effort of researchers in these fields. As such, it gave us the unique opportunity of establishing a real collaboration between expert scientists from different fields of interest in Israel and the USA. Both laboratories were linked by e-mail, so that ongoing communication occurs very often, to exchange ideas, results and methods. Additionally, two of the Israeli collaborators (S. Philosoph-hadas and S. Meir) have met in July 2001 with the American collaborator (P.B. Kaufman) during a Gordon Research Conference on Gravitropism in the USA, for coordination of the project. This enabled promotion of the project objectives in various aspects of the proposed research area and related subjects. Both synergistic and complementary modes of cooperation were employed in this project as follows: Both labs worked on the same aspects of graviperception using the two systems of snapdragon spikes and oat shoot pulvini, in which the Israeli and American teams, respectively, have gained significant expertise. In this manner we could confirm a more general sequence of events occurring during graviperception and leading to shoot bending. A good example of this fruitful collaboration was the mutual exchange of ideas and techniques in various systems. Thus, the Ca^{2+} antagonists developed initially by the Israeli team in snapdragon were assayed by the American team in oat shoots, and the amyloplast analysis developed initially by the American team in oat was easily applied by the Israeli team to snapdragon. The collaborators have submitted recently a joint article related to these issues. In addition, the Israeli team has submitted a joint article on the actin CK with Prof. P.K. Hepler, who assisted in part of the CK studies.

A.3.4. List of Publications (only reviewed publications)

1. Halevy, A.H., Torre, S., Borochoy, A., Porat, R., Philosoph-Hadas, S., Meir, S. and Friedman H. (2001). Calcium in regulation of postharvest life of flowers. *Acta Hortic.* 543: 345-351.
2. Friedman, H., Vos, J.W., Hepler, P.K., Meir, S., Halevy, A.H. and Philosoph-Hadas, S. (2003). The role of actin filaments in the gravitropic response of snapdragon flowering shoots. *Planta* 216 (6): 1034-1042.
3. Philosoph-Hadas, S., Berkovits-Simantov, R., Friedman, H., Meir, S. and Halevy, A.H. (2003). Role of ethylene in modulating auxin action during the gravitropic response of cut snapdragon spikes. In: *Biology and Biotechnology of the Plant Hormone Ethylene III* (M. Vendrell, H. Klee, J.C. Pech and F. Romojaro, eds.), pp. 311-312. IOS Press, Amsterdam.
4. Chang, S-C, Cho, M-H, Kim, S-K, Lee, J-S, Kirakosyan, A, and Kaufman, PB. (2003). Changes in phosphorylation of 50 and 53 kDa soluble proteins in greviresponding oat (*Avena sativa*) shoots. *J. Exp. Bot.* 54: 1013-1022.
5. Chang, S-C, Kim, Y-S, Lee, JY, Kaufman, PB, Kirakosyan, A, Yun, H-S, Kim, T-W, Kim, S-Y, Cho, M-H, Lee, J-S and Kim S-K. (2004). Brassinolide interacts with auxin and ethylene in the root gravitropic response of maize (*Zea mays*). *Physiol. Plant.* 121: 666-673.