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(54) Title: PROCESS OF GROWING PLANTS UNDER HYPERGRAVITY CONDITIONS

(57) Abstract: The present invention provides a process for the germination and/or modulation of plant development using the inventive concept of subjecting plants to simulated hypergravity conditions as a way of increasing efficiency and speed of plant development and/or germination.

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Descriptive Report

Process of growing plants under hypergravity conditions

Field of the Invention

5 The present invention relates to simulated hypergravity-like or simulated hypergravity conditions in plant development modulation. More precisely, the present invention provides apparatus and process to induce the effects caused by the increase in gravity, being applied in the modulation of germination and/or post-germinative development of different species, for example, *Euruca sativa*
10 Milll. The process of the invention provides several advantages, such as the increase in germination rate, fast and efficient development, high growth rate (volume and mass) and high rate of secondary metabolite production in comparison to growing processes under Earth's gravitational force (1G).

Background of the Invention

15 Biological studies in space environment have increasingly received special attention from researchers. There are scientific reports showing that changes in gravity as well as changes in the activity of Earth's magnetic field may interfere in living organism development. Hypergravity is one of these
20 changes and can be defined as the increase of weight caused by either the enhancement of the mass of a given body or the acceleration of it (simulated hypergravity condition). It can be seen during launch and re-entry of a spacecraft in the Earth's atmosphere.

25 On Earth, gravity is present during plant evolution, being used by them to regulate their growth and development (Soga, 2004). Nowadays, two hypotheses try to explain the mechanism of gravity perception in plants. Starch-statolith hypothesis states that dense bodies of starch called amyloplasts, found in root cells, act as a gravity sensor for plants (Kiss et al, 1996; Kiss et al., 1998; Fitzelle, 2001; Aubay-Centis, 2003; Kiss et al., 1999). On the other hand,
30 protoplast pressure hypothesis, by observing genetically modified plants with few amyloplasts, asserts that gravity perception is caused by the weight of root

cell content (Caspar, 1989; Guisinger, 1999; MacCleery and Kiss, 1999 Soga et al., 2004; Soga et al., 2005). However, until now, most data referring to gravity influence in plant physiology and morphology have been obtained from plants subject to changes in gravitational vector orientation (Aubay-Centis, 2003). For
5 this purpose, experiments in space provide microgravity conditions, while experiments based on centrifuges provide hypergravity simulated conditions (Soga, 1999).

Experiments under simulated hypergravity can represent the mechanisms involved in animals or plants, in tissues or cells in response to the
10 increase in the gravitational force (van Loon et al., 1993). In the same way, it is possible to create on Earth, by using human centrifuges, a hypergravity environment similar to those generally found in space missions or during an abrupt maneuver of a high performance aircraft. In plants, hypergravity produced by centrifugation is used to analyze the responses of plant seeding to
15 gravity stimulus (Hoson, 2002), although this technique has been employed in the separation of cell components, but only as a primary stimulus (Russomano et al., 2007). Gravitational forces greater than 1G have been useful for studying gravity role in plant growth (Kasahara et al., 1995). Experiments carried out by Hoson (2002) showed that hypergravity produced by centrifugation increased
20 cell wall stiffness due to gravitational force resistance (Soga et al., 1999; Soga, 2004; Hoson et al., 2002). In the same way, it was noticed growth inhibition of elongation in mustard epicotyles (Waltron and Brett, 1990), radish and cucumber hypocotyles (Kasahara et al., 1990), cress hypocotyles (Hoson et al, 1996), azuki bean hypocotyles (Soga et al., 1999), corn coleoptiles and
25 mesocotyls (Soga et al, 2003) and *A.thaliana* inflorescence stems (Tamaoki et al., 2006) in response to hypergravity. These results suggest that growth inhibition is due to the reduction of cell wall mechanical extensibility (Soga et al., 1999; Hoson et al., 2002; Soga et al., 2003; Soga et al., 2004).

However, morphological experiments carried out by the present inventors
30 showed that arugula seeds germinate faster when exposed to simulated

hypergravity. Such results are amazing considering the State of the Art and constitute the starting point to the present invention development.

Scientific and patent literatures regarding publications that are only partially related to the subject of the present invention, however, do not
5 anticipate or suggest, even indirectly, any of the objects of the present invention.

The article entitled "Simulated microgravity and hypergravity attenuate heart tissue development in explant culture" reports the study about hypergravity influence on heart tissue morphogenesis.

10 United States patent US 6,008,009, owned by Universities Research Association and entitled "Centrifuge-operated specimen staining method and apparatus" describes a method and an apparatus of preselected staining where the liquid stained reagents are applied and removed from the staining chamber.

International patent application WO 00/30718, filed by Arthur Kreitenberg
15 and entitled "Exercise apparatus involving centrifugal forces", describes an exercise apparatus involving centrifugal forces where centrifugal acceleration and Earth gravitational acceleration are summed.

European document EP1030554, owned by Oceaneering International, Inc. and entitled "Method and apparatus for cytoplasmic loading using an
20 impact-mediated procedure" describes a method and an apparatus for the introduction of macromolecules into the cytoplasm of living cells by an impact-mediated procedure that compresses cells with a predetermined number of solid particles in a blast of propellant gas. This procedure can be changed by gravitational conditions and is preferably carried out under hypergravity
25 conditions.

United States patent US 3,882,634, by NASA, describes a rotary plant growth accelerating apparatus based on rotation and translation motions about horizontal axes in order to administer nutrients to plants during rotation. Horizontal planetary path *reduces* gravity effects, that is, it simulates
30 *microgravity* effects, accelerating plant growth.

United States patent US 3,911,619, by Gravi-Mechanics Co., describes an apparatus for seed sprouting also based on rotation motions about horizontal axes. Horizontal planetary path *reduces* gravity effects, accelerating seed sprouting, and avoiding problems associated to gravity.

5 United States patent US 3,973,353, by Gravi-Mechanics Co., describes a different apparatus to accelerate plant growth based on the application of rotation and translation motions about horizontal axes in order to administer nutrients to plants during rotation. Horizontal planetary path *reduces* gravity effects, accelerating plant growth.

10 Results obtained by the present invention system and process are amazing from scientific and patent literature point of view.

Summary of the Invention

It is an objective of the present invention to provide a process of plant
15 growth and development under simulated hypergravity conditions. Therefore, the invention consists of an apparatus and a process that simulate the exposure to a hypergravity environment, which increases plant germination and/or post-germinative development.

Another objective of the present invention is to provide a process for
20 large-scale plant growth in a system that supplies hypergravity simulated environment.

Yet another objective of the present invention is to provide a process for large-scale plant growth that presents accelerated cell growth and cell differentiation.

25 Another objective of the present invention is a process for large-scale plant growth, which has a significantly greater growth (volume and mass) and a faster differentiation rate for an equal period in comparison to others under Earth's gravity.

These and other objectives of the present invention should be evident
30 and valued from the detailed description of the invention.

Brief Description of the Figures

Figure 1 shows individual development of *Euruca sativa* Mill subject to two different environments, a simulated hypergravity environment (+7G_z - Figure 1A) and Earth's gravity environment (1G - Figure 1B), used as control.

5 Tests were carried out during an intermittent period of four days.

Figure 2 shows the comparison of arugula growth in soil method.

Figure 3 shows the difference between the growths obtained in paper with water-centrifuge and soil-control.

10 Figure 4 shows a histological cut of a plant subject to simulated hypergravity.

Figure 5 shows another histological cut of a plant subject to simulated hypergravity.

Figure 6 shows a histological cut of a plant not subject to simulated hypergravity (control).

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Detailed Description of the Invention

From the detailed description of the present invention, those professionals familiar with the subject will immediately identify its great technical and economic value. For the purposes of the present invention, the term "hypergravity" is defined as that resulting from conditions capable of inducing the effects caused by the increase in the gravitational force, using hypergravity-like or simulated hypergravity situations (in this case, rotation). The examples described below aim to provide ways of reproducing the invention, but they should not be interpreted as a limitation to the invention nor the only way of accomplishing it.

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Example 1

A plastic pot with a diameter of 60 mm and height of 62 mm, with 44 g of dark soil (Mumosoto, a kind of humus) and 10 seeds of *Euruca sativa* Mill, (KAD type, with increased moisture) was put in each arm end of a centrifuge, adjusted to rotate at 92 rpm, in order to provide a +7G_z environment. Seeds mentioned above were subjected to this condition during intermittent periods - in this case

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from 8 AM to 5 PM, totaling 9 h/day for four consecutive days. At night, hypergravity action was removed, that is, from 5 PM to 8 AM next day plants remained under Earth's gravity force (1G). Pots were closed with a plastic coverage to avoid water evaporation by forced convection resulting from the spinning. Pots were safely fixed to the arm ends of the centrifuge with a metal bolt. Thus, during rest the pot opening remained at a 90° angle to the centrifuge arm. During centrifuge rotation, the pot remained at 0° angle to the centrifuge arm, simulating an increase of Earth's gravitational force in the z axis. A 3-millimeter hole was made on the side of each pot to allow ventilation. Two similar plastic pots were kept open in the same room to be used as control (1G). Room temperature was adjusted to 22°C, and water (0.5 mL volume) was added to the pots before and immediately after the experiment. Experiment was carried out twice in order to verify the reproducibility of it.

Results showed that the seeds exposed to +7G_z germinated in three days in comparison to the four days necessary for the seeds in the control pots. At the end of the process, plants subject to simulated hypergravity were removed and measured by a caliper rule from root to top. Two embodiments of the invention process were:

(i) in the first embodiment, the mean height of the plants subject to +7G_z was 3.2 cm, against a mean of 1.9 cm of control plants, showed in Figure 1;

(ii) in the second embodiment, when more plants were subjected to the invention process (n=14), the mean height of the plants subjected to simulated +7G_z was 2.2 cm, a significantly greater value than the development of plants under 1G (control), which was 1.8 cm (p=0.02). Results described above support the applicability of the present invention process in the modulation of plant germination and/or growth.

Although the technical reasons for such amazing results are not totally elucidated, some guidelines to technical debate could explain such results. One of them could be related to the influence of plant hormone auxin, which modulates plant growth. Term auxin comes from Greek "auxein", that means to grow; therefore, auxin is characterized by being able to induce cell elongation in

stem sub-apical region. Auxin is a vegetal hormone responsible for plant cell growth, stem and root elongation, and fruit development. It is well known that auxins control gravitropism, promote apical dominance, and retard abscission. Auxin also affects physiologic processes, including phototropism, gravitropism, fruit development, among other functions. However, elongation is one of the most important effects of auxin action. One important stage of elongation is cell edge acidification caused by an electrochemical gradient, which leads to proton secretion through plasmatic membrane, promoting cell wall acidification, resulting in enzymatic activity increase. This enzymatic activity increase promotes cell structure ductility, enabling cell elongation. When water penetrates cell, osmotic pressure forces expand it. The amazing and significant development of *Euruca sativa* Mill after four days of exposition to intermittent simulated hypergravity could therefore derive from the variation of the amount of auxin. Likewise, hypergravity may change auxin levels in tissues. If so, it can be deduced that changes in the components of secondary metabolism may happen, such as enzymatic activities of phenylpropanoid and terpenoid pathway, as well as in the synthesis of development regulatory molecules.

Example 2

From the results obtained in Example 1, new experiments were carried out. In the present example, only soil method was employed, despite the variances obtained from the water factor. At the end of this experiment, however, it was visually noticed that the soil of the samples in the centrifuge was dry on the surface, possibly negatively influencing plant growth. Difficulty in establishing the exact amount of water that should be used to have the same moisture under 1G_z as well as under +7G_z was evident. Figure 2 shows that control sample growth (3.76 ± 1.21) was statistically greater ($p = 0.00$) than centrifuge sample growth (3.15 ± 1.00). However, when comparing growths in paper with water-centrifuge of Example 1 experiments (8.44 ± 1.43) to soil-control (3.76 ± 1.21), a significant difference ($p = 0.00$) was noticed, as shown in Figure 3. Therefore, the last experiment aimed only at the application of

paper with water method in order that the water factor did not interfere in results.

In the following experiment it was obtained a greater growth in centrifuge samples (4.00 ± 1.01) with statistically significant values ($p = 0.00$). However, plants did not reach 1 cm of difference from control (3.49 ± 1.27). In both samples, less growth was noticed, because mean height did not exceed 4.5 cm, which differs from previous experiments, when mean height was up to 8.5 cm. Factors that could have influenced results were low temperature and moisture conditions.

In the present invention, total phenolic compounds were quantified in both conditions and were not significantly different ($p = 0.06$), showing, therefore, that the hypergravity simulation did not affect the phenol production in plants (Table 2).

Table 2 – Values of total phenols in gallic acid equivalents (mg/g)

Control	Centrifuge
0.08538	0.08850
0.06914	0.07852
0.05898	0.08928
0.06152	0.07912
0.07970	0.07872
0.06758	0.07952
0.05172	0.07150
0.08830	0.07932

Finally, in the histological cut of arugula cotyledons a difference in its essential oil distribution was noticed. In cotyledon-centrifuge cell, oil was distributed in the whole cell membrane, and small oil drops spread in cytoplasm were noticed (Figures 4 and 5). On the other hand, in cotyledon-control cells, oil was located in the center of the cell, showing a single, big drop (Figure 6).

By means of preliminary studies carried out at Microgravity Center – Pontifical Catholic University of Rio Grande do Sul, it was concluded that *Eruca*

sativa seed germination and growth are greater when these seeds are under intermittent simulated hypergravity conditions.

Results presented suggest that plant growth and/or development under simulated hypergravity can be an alternative to minimize one of the main problems confronted by agribusiness and pharmaceutical and cosmetic industry. In a shorter period and with low operational costs, it is possible to have an increase of vegetal raw production, as well as to provide quantitatively standardized vegetal extracts. In the invention process, simulated hypergravity level, duration of exposure and/or intervals are chosen according to the intended result of plant growth/development.

Those professionals familiarized with plant growth and/or germination will immediately value achievements and identify several technical, economic, environmental and health advantages of the technology here described. Small variations in the way of carrying out the invention here described should be considered within the scope and spirit of this invention and its claims.

Claims

Process of growing plants under hypergravity conditions

1. Process of growing plants **characterized** by comprising at least one
5 step of submitting the vegetal material to hypergravity conditions, resulting in a modification of the development of said plants.
2. Process, according to claim 1, **characterized** by the fact that said hypergravity condition is provided by a centrifuge.
3. Process, according to claim 1, **characterized** by the fact that said
10 hypergravity condition is applied during intermittent periods.
4. Process, according to claim 1, **characterized** by the fact that the hypergravity level, duration of exposure and/or intervals are chosen according to the intended result of plant growth/development.
5. Process, according to claim 1, **characterized** by the fact that said
15 hypergravity condition is applied to seeds, modulating their germination and/or posterior development.
6. Process, according to claim 1, **characterized** by the fact that said hypergravity condition is applied to previously germinated plantules, modulating their development and/or metabolism.

Figures

Figure 1

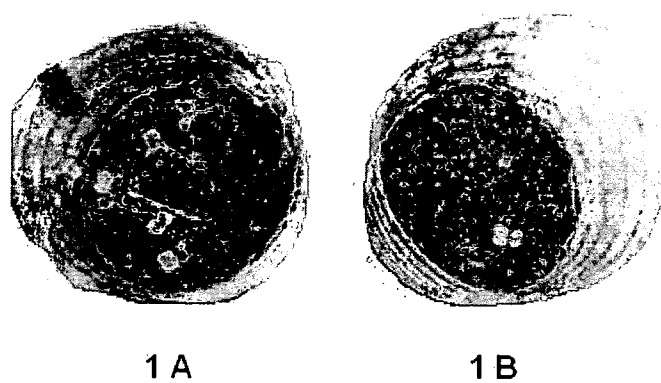


Figure 2

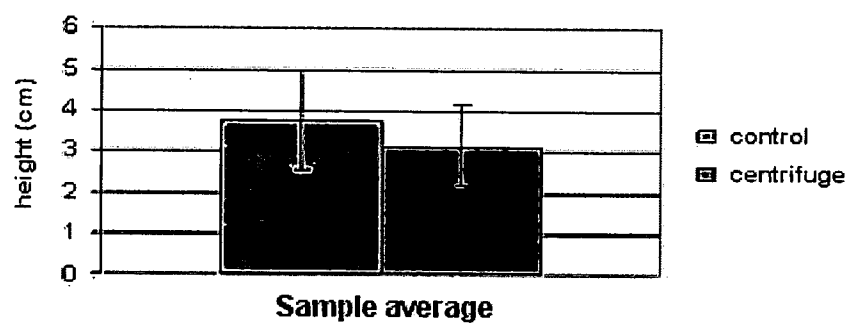


Figure 3

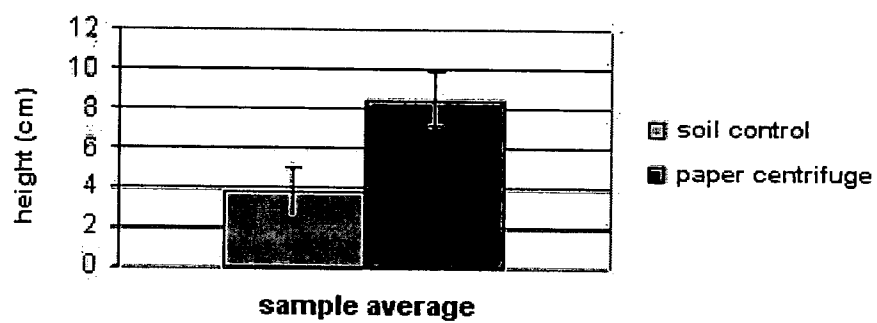
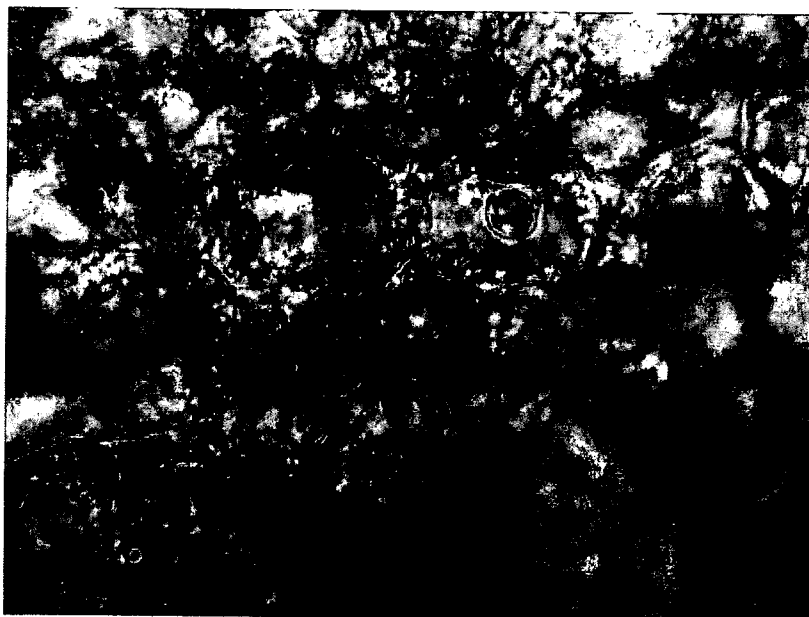


Figure 6



INTERNATIONAL SEARCH REPORT

International application No.
PCT/BR 2008/000199

A. CLASSIFICATION OF SUBJECT MATTER

IPC⁸: **A01H 3/00** (2006.01); **A01C 1/00** (2006.01); **A01G 7/00** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC⁸: A01H3/00; A01C1/00; A01G7/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC, WPI, TXTE, TXTG, NPL, EMBASE, XPESP

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Russomano et al. "Effects of simulated hypergravity on biomedical experiments"; IEEE Engineering in Medicine and Biology Magazine; Vol 26 (3) 66-71: 1 May 2007 (01.05.2007) <i>Abstract; Page 67 Column 2 Lines 1-4, Page 68 Column 2 Lines 29-35, Page 70 Column 2 Line 42 - Page 71 Column 1 Line 12</i> --	1-3, 5, 6
X	Soga et al. "Hypergravity inhibits elongation growth of azuki bean epicotyls independently of the direction of stimuli" ADVANCES IN SPACE RESEARCH; Vol 36 (8) 1269-1276: 1 August 2005 (01.08.2005) <i>Abstract; Description Page 1269 Column 1 Line 10 - Column 2 Line 4, Page 1270 Paragraph 2.1., Discussion Page 1273</i> --	1, 2, 6

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
27 October 2008 (27.10.2008)Date of mailing of the international search report
11 November 2008 (11.11.2008)Name and mailing address of the ISA/ AT
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Dresdner Straße 87, A-1200 ViennaAuthorized officer
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Continuation of first sheet

Continuation No. II:

Observations where certain claims were found unsearchable

(Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:

Claims Nos.: 4 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Process parameters cannot be given by the intended or desired result. Process parameters of a centrifugation have to be specified by revolutions per minute (RPM) or the g-force and the time period.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/BR 2008/000199

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Matsumoto et al. "Up-regulation of expression of tubulin genes and roles of microtubules in hypergravity-induced growth modification in Arabidopsis hypocotyls" ADVANCES IN SPACE RESEARCH; Vol 39 (7) 1176-1181: 1 July 2007 (01.07.2007) <i>Abstract; Page 1177 Paragraph 2.1. Lines 1-10, Page 1179 Paragraph 3.2. Lines 1-3, Discussion Page 1180 Column 2 Lines 5-20</i>	1, 2, 6
X	-- Toyota et al. "Hypergravity stimulation induces changes in intracellular calcium concentration in Arabidopsis seedlings" ADVANCES IN SPACE RESEARCH; Vol 39 (7) 1190-1197: 1 July 2007 (01.07.2007) <i>Abstract; Introduction Page 1190 Column 2 Line 5 - Page 1191 Column 1 Line 3, Paragraph 2.4. Lines 1-4, Page 1192 Paragraph 3.1. Lines 7-10, 28, 29, Page 1193 Column 2 Lines 30-34, Page 1196 Discussion Column 1 Lines 13-20</i>	1-3, 6
X	-- Wakabayashi et al. "Changes in levels of cell wall constituents in wheat seedlings grown under continuous hypergravity conditions" ADVANCES IN SPACE RESEARCH; Vol 36 (7) 1190-1197: 1 July 2005 (01.07.2005) <i>Abstract; Page 1293 Introduction Column 1 Line 17-36, Paragraph 2.1. Lines 1-7, Page 1294 Paragraph 3. Lines 1-20, Page 1295 Paragraph 4. Lines 1-15, Page 1193 Column 2 Lines 37-42</i>	1, 2, 5
