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黄金大米现状

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摘 要 迄今为止，黄金大米的研发和上市已经超出了当初预期的时间。事实证明，黄金大米有潜力缓解一个困扰数百万人的重要公共健康问题——维生素 A 缺乏。根据高度的预防性原则（已被证明是没有必要的）制定的联合国卡塔赫纳生物安全协议书阻遏了科学发展和科学合作，尤其延误田间的表型筛选。到目前为止，黄金大米仍未能帮助克服维生素 A 缺乏症，以及由维生素 A 缺乏症造成的本可预防的失明和死亡。25 年来，维生素 A 缺乏一直被联合国列为重要的公共健康问题。当然，发明家的最初梦想——贡献这项技术来帮助资源匮乏而又希望从该项技术中获益的人们——在慈善机构和公共部门持续的资金支持下，依然是坚定的，且必然会实现。

关键词 黄金大米；维生素 A 缺乏；先正达公司；国际水稻研究所；卡塔赫纳生物安全协议；营养；公共健康；水稻；联合国儿童基金会；联合国；世界卫生组织

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1 维生素 A 缺乏

维生素 A 缺乏（VAD），影响着 1 900 万孕妇和 1.9 亿学龄前儿童的健康，尤其是在非洲和东南亚国家[1]。维生素 A 缺乏会导致每年大约有 50 万例儿童失明[2]。在没有治疗的情况下，约一半的儿童会死亡。最近，VAD 已成为公认的营养获得性免疫缺陷综合征[3]，它可导致每年 100 万到 200 万的人死亡，主要是幼儿和一些母亲。受 VAD 影响的大多数人，如果其免疫系统正常，就不会引起失明甚至死亡（与 R Russell 的个人交流）。这种严重的死亡率在 2010 年[4]超过了全球由艾滋病毒/艾滋病、结核病或疟疾引起的死亡率[5]。

5 岁以下儿童死亡率居高不下和重度贫困都与维生素 A 缺乏症密切相关。在印度，5 岁以下儿童的死亡人数每年超过 200 万，比其他国家的情况更糟糕，这可能与饮食多样性的局限性以及维生素 A 补充剂的低覆盖率有关，因此，1980 年至今，印度在降低死亡率方面的进展非常缓慢[3]。

维生素 A 缺乏在中国的城市和农村也是一个常见的营养问题。2004 年，3 岁到 12 岁儿童中维生素缺乏患病率是9.3%。其中城市为3.0%，农村地区为 11.2%。轻度维生素 A 缺乏症的患病率占总人口的 45%，其中 29%在城市地区，50%在农村地区[6]。2006 年的一项调查发现 VAD 影响了中国 12.2%的 0 至 6 岁的儿童，其中 0.5%的儿童深受重度 VAD 的折磨。生活在中国西部地区的儿童，如果他们的母亲教育程度低或是少数民族，那么这些儿童患 VAD 的风险高[7]。回顾截至 2005 年的

图 1 左眼失明的年轻印度女子（不过她还是幸运的，因为大多数维生素 A 缺乏症患者在孩童时期就死了）

Fig. 1 This Indian young woman, blind in her left eye, is nevertheless lucky, most vitamin A deficiency sufferers die as young children

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10 年内数据，2009 年世界卫生组织公布的数据表明：在中国学龄前儿童或者母亲中夜盲症（维生素 A 缺乏症的早期临床症状）患病率较少；学龄前儿童 VAD 有轻度发病率；而 VAD 在中国对孕妇来讲是一个严重的公共卫生问题[8]。

“尽管增加食用维生素 A 丰富的食品似乎是一个合理的解决方案，但实际上对贫困家庭的学龄前儿童而言，仅仅依靠饮食满足维生素 A 的摄取要求是非常困难的。富含维生素 A 的动物性食品，如动物肝脏、鸡蛋、奶酪、奶油，这些往往超出了贫困家庭的消费能力。另一个使学龄前儿童很难满足他们的膳食中维生素 A 需求的关键因素是水果和蔬菜来源的维生素 A 的生物利用度不高。一个年龄 1 岁到 3 岁的儿童每天需要吃 8 份深绿色叶子类蔬菜才满足所需的维生素 A 摄入量。植物性食物中维生素 A 的低生物利用度带来的现实是‘对于大多数家庭贫穷的儿童来说，实际上是不可能仅仅通过蔬菜和水果摄入来满足维生素 A 的需求’。植物性食物来源的维生素 A 生物利用度低，在某种程度上可以解释为什么在那些深绿色叶类蔬菜和其他富含维生素 A 的植物供应充足的地区仍有儿童患有维生素 A 缺乏症”[3]。

抛光后的白米基本上只含有碳水化合物，不会有利于储存。它基本上只包含提供能量的碳水化合物，而缺少生命必需的矿物质和维生素。因此，维生素 A 缺乏是一个全球普遍的问题，尤其是在世界大部分以大米为主食的地区，而且这些国家贫困人口的 80％或者更多的卡路里来源于大米（与 H Bouis 的个人交流）。

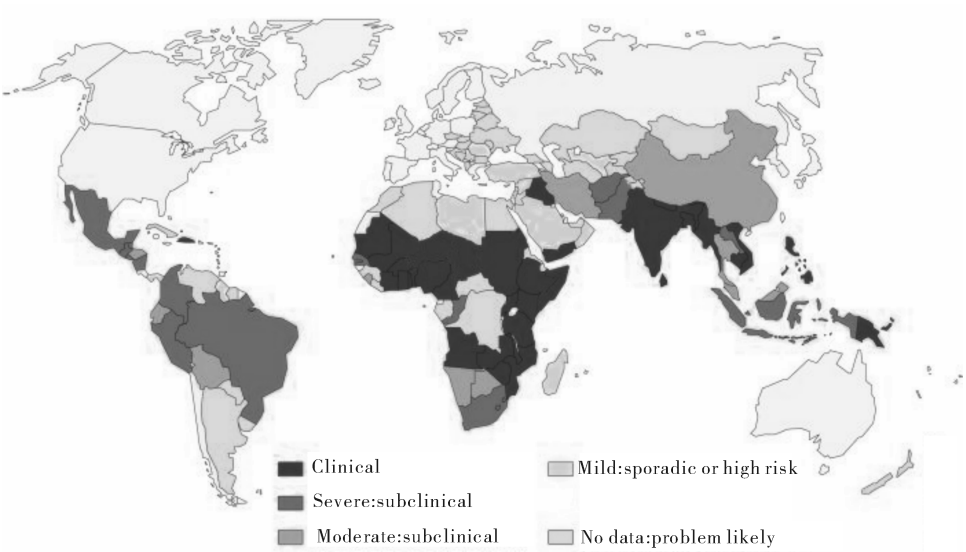

图 2　维生素 A 缺乏在各国公共卫生中的重要性

Fig. 2　Public health importance of vitamin A deficiency, by country

2　缓解 VAD 是可能的，负担得起的，但是很棘手

在 1990 年联合国世界儿童峰会上，150 多名政府首脑和高级政府官员承诺他们的政府将致力于到 2000 年真正消除维生素 A 缺乏症及其所引发的后果[9]。这个承诺在 1992 年联合国营养国际会议上得到加强，指出解决维生素 A 缺乏症是政府可以追求的最具成本效益的保证儿童健康和生存的策略。会议得出的结论是社会各界应该运用多种策略来从根本上消除维生素 A 缺乏症。这些策略应该包括母乳喂养宣传、饮食多样化、维生素 A 的补充剂，以及食品强化[10]。

2003 年联合国儿童基金会和微量营养素倡导组织以“控制维生素和矿物质的缺乏——一个可负担的机会——提高 20 亿人的生活和加强经济发展的脉搏”为标题，发布全球“维生素和矿物质缺乏现状”的进展报告。“也许在现在，没有现成的其他技术能够提供如此大的机会来用如此低的成本改善生活，加快发展”[11]。

3 黄金大米

生物强化这个术语在之前的联合国会议上并没有被提出来。然而这项研究其实早在20世纪90年代初期，就由因目标一致而达成共识的Ingo Potrykus和Peter Beyer团队开展了。该研究是1999年名为“黄金大米”的研发项目的基础[12]。黄金大米是第一例有目的性创建的以生物强化为目的的粮食作物，专门为了解决维生素A缺乏症而设计。

图3 Ye等2000年描述的“黄金大米”的雏形

Fig. 3 The prototype “Golden Rice”, described by Ye et al, 2000

生物强化类农作物指经由任何方法，包括在需要时采用的遗传工程手段，来实现育种目的的农作物，从而增强微量元素（维生素或矿物质）含量或者生物利用度。预计这种方式会比利用补充剂（‘维生素药片’）或者强化剂（将矿物质或者维生素加入到加工过的食品）更为便宜、更具有可持续性和获得更大的成本效益，从而解决微量元素不足[5]。

2000年，黄金大米第一次在《时代周刊》的美国版（图4）和亚洲版上广泛宣传。

图4 2000年9月《时代周刊》

Fig. 4 Time magazine, September 2000

那么黄金大米工程取得了怎样的进展，项目当前的状态又如何呢？

4 黄金大米工程的愿景

全球黄金大米人道主义工程的愿景是黄金大米创造者开始他们研究时的初衷：提供给那些发展中国家的贫困的大米消费者，让他们的主食大米中就含有不用花钱的维生素原，这是他们需要的且可以从中受益的。

1999年3月5日，发明家就他们研发的营养技术提出专利申请。随着生物强化概念被成功验证，菲律宾国际水稻研究所（早期就关注到了该技术的升值潜力）要求洛克菲勒基金会对该技术进行知识产权（IP）审计。洛克菲勒基金会将这项任务交给了国际农业生物技术应用服务组织（ISAAA），接着该任务转包给了ISAAA的执行秘书所领导的康乃尔大学的一个研究小组。

尽管有了解他们这项发明的公司曾与发明者们联系，但他们不为所动。相反，发明者们公开表示，计划将这项技术免费赠送给需要它的人。然后，该项目的负责人坚持认为——由于此前有一个与之不相关的材料转让协议中也涵盖了一个用于黄金大米的技术——该营养技术专利应该由他的公司来管理而不是发明家。由于负责人不妥协，讨论受阻。2000年2月20日，发明家将相关专利和他们所有的权利转让给了弗莱堡大学的生物技术公司——Greenovation公司。

几乎同时，捷利康农药（其后来与诺华公司合并为先正达公司）为了技术的专利权联系了发明家，之后就直接与Greenovation公司联系。根据要求，Greenovation公司在2000年4月14日及时批准了捷利康公司对该技术的专有权：该专利在人道主义应用中将为免费，但用于商业应用则会收取专利费。在2000年4月14日这一天，为了实现发明者们希望发展中国家贫困农民可以免费得到该技术的初衷，捷利康公司又将该技术的使用权授予了发明者们。通过创建这种公私合作伙伴关系，发明家将该专利技术的商业权利给了捷利康公司，以回报公司对发明家的人道主义愿景的支持。Beyer博士评论说：“捷利康公司是全球唯一一家在研究植物中类胡萝卜素的生物合成研究中久负盛名的公司，因此，捷利康公司是我们的天然合作伙伴”。捷利康公司在新闻发布会上解释说“这项技术，以及转化的水稻种

子，将在严格管理下，根据需求提供给发展中国家的国际和国家研究机构，进而协助在当地的应用，完成水稻品种生物安全及其他评估。当当地政府对品种完成人与环境的安全评估并审批通过后，黄金大米的种子可以经由常规育种方式来繁殖，并且分发给资源匮乏的农民种植、收获、展开小规模的商业活动（邻居和本地市场）和消费。”

这种合作将免费提供黄金大米或者相关的技术给国际和国家研究机构，这些科研机构有权将具有该性状的水稻种子分发给贫穷的农民。即使水稻种子在本地商业化销售，该性状仍须是免费提供的。

捷利康公司进一步表示，“我们也会支持必要的生物安全及风险评估工作，我们最近正在和我们在日本的旗下公司 Orynova 就改良传统品种（例如 IR64）展开讨论。”

康奈尔大学发表的关于黄金大米知识产权的研究[13]的文章对该项目毫无益处。在 2000 年 5 月 31 日 Potrykus 教授给第一作者的邮件里指出：“你的分析已经导致了一个可怕的未来：如果要实现自由运营黄金大米，实现这个面向 78 个水稻种植国家的人道主义工程，就需要向 32 个专利持有者提出申请并获得他们的同意”。捷利康实现发明家初衷的第一步就是完成了一个合理的知识产权审计，表明实际上只有极少数的几项专利可能在该项目中被侵权，并且取得了这些知识产权持有人的同意，让他们的专利在这个具有明确的人道主义意义的项目中被免费使用[14-15]。

捷利康公司和发明家协议规定，无论任何一方对技术进行改进，双方都要交叉授权许可。（几年后，当 Greenovation 试图通过风险投资家来资助他们的医药生物技术战略时，由于农业生物技术在欧洲存在争议，风投公司要求在他们投资前 Greenovation 放弃维生素 A 前体技术方面的商业项目。Greenovation 向先正达公司寻求了帮助，由先正达购回剩余的相关专利。）

通过这些机制，发明家得以将他们的发明捐赠给世界资源匮乏的地区，使在水稻品种方面的营养技术可以免费提供，从而使人们从中获益。特别强调的是，参与黄金大米研发的任何人都不会从它的使用中获取经济利益[5]。

4.1　2000 — 2005 年

发明家和捷利康公司达成最初的协议后，很快的，当然也非常巧合，英国农业企业捷利康公司和瑞士诺华公司的农业商业部门合并成了先正达公司，该公司总部位于瑞士。1999 年 12 月 2 日，捷利康公司员工得知了合并的消息。2000 年 1 月，《科学》上发表了 Potrykus 和 Beyer 团队在白色水稻胚乳中生产 β-胡萝卜素的重大突破[12]。2000 年 11 月 13 日，先正达公司进入纽约、伦敦和苏黎世股票市场，2001 年 1 月 20 日，Potrykus 和 Beyer 团队同意先正达公司取代捷利康公司。2001 年 4 月，我作为先正达公司分管兼并与收购、风险投资和知识产权的全球主管，获得了瑞士的工作签证，并且居住在瑞士，意外地能够很方便地与居住并工作在附近的 Potrykus 和 Beyer 教授联系。

在 2001 年的一个新闻发布会中，黄金水稻中 β-胡萝卜素含量水平被绿色和平组织指责在缓解维生素 A 缺乏中是毫无意义的。当时，已经反对转基因作物 5 年的绿色和平组织在发布会中说，一个哺乳期的妇女每天要吃 18 kg 煮熟的黄金大米才能补充足够的维生素 A。当时没有人知道黄金大米中类胡萝卜素生物利用率的情况，所以其实没有人有能力做出该评判（之后该言论也被证明是错误的）。2001 年 2 月，绿色和平组织的 Charlie Kronick 在英国的《卫报》报道说“数十亿英镑花在了开发这种大米上，这是从更合理地解决维生素 A 缺乏的方式上转移资源”[16]。这只是第一个被那些反对者夸大其辞的例子。在同一期的《卫报》上报导了洛克菲勒基金会的 Gordon Conway 总裁加入反对 GMO 积极分子 Vandana Shiva 的队伍，他认为“黄金大米在使用公关这条路上已走得太远”[16]。据报美国电视台播放了由美国生物技术产业支付的广告，广告画面暗指黄金大米正在美国种植，这对欧洲那些早期就已进入黄金大米工程的国家是一个尴尬的惊喜（当然也有误导性）。

无论如何，热衷于在北美和欧洲进行商业开发，优化“功能性食品”方面技术的先正达开始了他们的研发，例如先正达在英国伦敦西部的布拉克内尔的 Jealott's Hill 国际研发中心的科学家和 Peter Beyer 博士在弗莱堡大学的实验室之间的合作项目，目的是探讨对黄金水稻雏形的改良。

这个志愿性质的黄金大米人道主义委员会最初是被邀请去给发明者提供建议和指导如何面对可能遇到的道德挑战。2000 年 8 月 18 日，在英国芬赫斯特的捷利康公司的第一次委员会会议中形成了使命宣言。其中一部分是这样表述的：“人道主义委员

会认为黄金大米在减轻发展中国家中营养不良人群在维生素A缺乏方面有潜力成为一个有价值的工具。人道主义委员会同时也认为,在黄金大米的授权方面应谨慎,但是应加快开展其在当地的环境安全、人类健康安全和社会效益的评价工作。”

议程之一是听取捷利康公司生物安全管理专家关于转基因作物中分子特性方面的建议,以确保产品依据2000年提出的卡塔赫纳生物安全协议书(尽管在2003年尚未正式执行)可以注册应用。在这次会议上还建议,基于卡塔赫纳协议,为了实现面向全世界的使用,应选取一个唯一的黄金大米转化事件,与通过常规育种的方式导入到哪种水稻品种无关。

同时,随着黄金大米人道主义委员会的成立以及按照先正达和发明家之间的协定[5],水稻研究机构公共部门的网络开始逐渐形成并迈向实现发明家们愿望的道路。第一个是菲律宾的国际水稻研究所,主管Ron Cantrell博士与Potrykus教授签署了一个使用许可协议,该协议的生效日同Potrykus和先正达之间的协议生效日一样,都是2001年1月20日。2001年1月22日黄金大米样品由发明家和我一起交付给了国际水稻研究所。黄金大米种子由发明家亲手交给国际水稻研究所的时候,实际上卡塔赫纳议定书尚未执行。Cantrell在一次国际水稻研究所、洛克菲勒基金会和先正达的新闻发布会中说到:“国际水稻研究所获得黄金大米初始样品标志着我们的工作迈出了重要的一步,这样使得我们终于可以开始使用当地的水稻品种进行所需的测试了。我们预期国际水稻研究所在正在进行的‘黄金大米’研究工作中将发挥重要作用,并会将其带给世界上数百万的水稻种植者和消费者。菲律宾的国家水稻研究所和在孟加拉国、中国、印度、印度尼西亚、南非和越南的类似国家机构也进入到了该项目中。”

2002年9月21日在北京召开的第5次人道主义委员会会议中,委员会要求获得特许的机构团体利用Beyer教授团队成功研发的转化载体再培育1 000个的转化事件,希望从中挑选出一个更好的转化事件,在之后所有的黄金大米授权机构中应将其作为唯一的转化事件进行后续改良。

2003年3月24日,人道主义委员会成员之一,也是前育种专家和世界粮食奖获得者Gurdev Khush博士在一封电子邮件中说:“背景干净的水稻品种IR64具单拷贝的维生素A基因不需要进一步的转化工作,它和其他的水稻品种一样。然而田间测试时,它的产量潜力和许多形态特征方面的改变应该进行评估”。

直到2003年4月,在苏黎世召开的第6次委员会会议上,公共研究机构团体并没有获得新的转化事件。然而在苏黎世会议上也有令人兴奋的消息,先正达公司子公司Orynova、荷兰的Mogen BV和日本烟草公司在黄金水稻改良方面取得了进步,且后者拥有了选择标记移除的新技术。

日本做了大约800个SGR1转化事件。10个具有单一插入位点,良好的叶色表型,同时消除了选择标记基因的转化事件被筛选出来,T_2代植株在英国种植。最好的转化事件表现出了13 μg/g的总类胡萝卜素含量,而之前验证的黄金大米总类胡萝卜素含量为1.6 μg/g。欧盟监管机构对这方面也产生了兴趣,开展了关于T_4代植株在欧盟和美国的田间试验的讨论。另外,在Peter Beyer实验室也完成了大约200次的转化事件。这样总共就有1 000次转化事件,其中先正达公司的4个和Freiburg/ETH/Cu Long Delta(越南)水稻研究所Beyer弗莱堡实验室的Hoa博士的2个转化事件被选择用于田间试验。

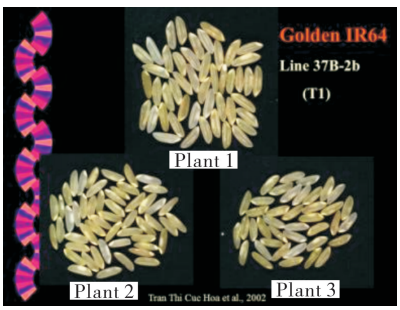

图5 Cu Long水稻研究所(越南)的Hoa博士在德国弗莱堡Beyer教授的实验室转化IR64获得的黄金大米

Fig.5 Golden Rice IR64 transformed by Dr Hoa of Cu Long Rice Research Institute, Vietnam, in Prof Beyer's Lab in Freiburg, Germany

2003年10月8日,Potrykus博士在给黄金大米研发网络的一封电子邮件中写道:黄金大米的田间试验在美国和西班牙正处于“规划阶段的后期……并且我们希望孟加拉国、印度、菲律宾和越南也会开展田间试验……今年早些时候安南(联合国秘书长)在联合国水稻年上宣布,这些试验可以产生农艺性状表型和性状稳定性的数据,并且增加2004年可收获的水稻种子。……这是一个巨大的挑战,

需要我们这个机构网络中拥有必要的专业知识的成员的参与……该工作也将因你们与当地监管机构的互动而保证其在符合规定的前提下尽快完成。……越南和印度已经有了黄金大米 IR64 的种子。在 1 月份(计划中)会议之前我们还将争取将维生素 A 表达水平高的先正达种子安排在同一试验计划内，并将 IR64 黄金大米分发给孟加拉国和菲律宾相关单位。……在美国，人道主义委员会成员 Robert Russell 博士和他的合作者已经完成了人体食用试验的计划和资金筹集方面的工作。为了获得 2004 年在中国能够开展试验的许可，包括取得伦理许可的工作正在积极进行中。”电子邮件还认为“鼓励在基础研究方面的发展，将最终带来改良的含有更高的 β-胡萝卜素含量的第二代黄金大米的出现。”

到了 2003 年 11 月 3 日，根据 Potrykus 博士的建议，国际水稻研究所招聘了一位黄金大米网络协调员——Gerard Barry 博士，由 USAID 提供资金。并且根据协议还需招聘项目经理来帮助委员会，该人选与先正达基金会资金一起预计于 2004 年初到位。(Jorge Mayer 博士于 2004 年到 2008 年担任该职位，当他因为家庭原因回到澳大利亚后，Potrykus 博士从先正达退休，从 2008 年到 2010 年担任该职位)。

为了筹划田间试验，Potrykus 教授 和 Swaminathan 博士发起了一个大型的筹备会议，该会议于 2003 年 12 月 15 日在印度德里举行，众多黄金大米计划的参与者们齐聚一堂。Barry 博士有精彩表现。

2003 年和 2004 年，先正达公司在生物技术管理方面面临着艰难的选择。先正达公司成立后，该公司前身的两个公司的所有的生物技术投资项目极为庞大，以至于不可能所有的项目能够得到足够的资金支持。如果能够丢弃一些投资潜力小的项目，使得资源得以释放，那么在那些具有更重要的商业前景的项目上才能够投入更多的资金，也就可能取得更大的进步。

Benedikt Haerlin，绿色和平组织在欧洲的反转基因运动的主导者，在 2001 年初发表声明：黄金大米对绿色和平组织构成了道德挑战，同时绿色和平组织不会破坏在菲律宾的黄金大米田间试验。无论如何，“近些年来，在转基因作物生产中的关于风险和道德方面的辩论已经变得相当有争议。在关于食物安全和环境这一关键问题上的讨论已经到了延迟甚至阻碍采用这项重要技术的程度”[18]。尤其是在欧洲大多数地区都是如此。在这个阶段，在没有任何公告的情况下，先正达公司决定放弃在黄金大米研发中的商业利益。它在法律约束力上仍然具有支持发明家的人道主义工程的义务，这在先正达正式员工间广受欢迎，同时在激励新成员加入先正达公司方面也有非常积极的作用。

世界其他地方对转基因作物的态度相对轻松。随着在印度批准的 3 种 Bt 杂交棉的种植，农业部部长 Rajnath Singh 在 2003 年 12 月 18 日宣布“在转基因项目网络中涵盖了 12 种作物。”“印度农业研究委员会(ICAR)的提议涵盖了玉米、木豆、鹰嘴豆、大豆、棉花、油菜、西红柿、茄子、香蕉、番木瓜、马铃薯和木薯”并且关注各种性状。甚至在 2004 年 2 月 11 日“在高级内阁部长包括英国外交大臣 Jack Straw 和环境部长 Margaret Beckett 之间达成了共识，认为政府应该给在英国的第一批转基因玉米开绿灯……尽管公众可能不太接受。”“Beckett 女士说没有任何科学证据表明应该彻底禁止种植转基因作物”[19]。

2004 年 1 月，Rachel Drake 博士，先正达英国 Jealott's Hill 国际研发中心的项目负责人在先正达公司作了内部通报，指出在先正达来自同一 SGR1 转化事件不同品种中，在不同时间间隔中，胡萝卜素的含量有所变化。也正如预期的那样，类胡萝卜素含量会逐步下降。“最新数据表明多个地点的田间试验在评价性能性状方面有极为重要的作用”。直到 2004 年 2 月 13 日，她仍在积极寻求我的批准，以经过内部必要的流程来捐赠、存档 SGR1 继而推进 SGR2。此进程的重要会议于 2004 年 3 月 31 日到 4 月 1 日在 Jealott's Hill 进行。

正如 2003 年 10 月 8 日 Drake 博士团队的一封电子邮件所预期的，到 2004 年 3 月为止，在 Potrykus 教授实验室基于新的高表达转化载体已获得了构建 30 个高 β-胡萝卜素含量的转化事件。这些就是著名的 SGR2 转化事件。

2004 年 9 月中旬，第八届黄金大米人道主义委员会会议在美国的路易斯安那州召开。令人非常兴奋的是大家首次亲眼目睹了在露天田间环境下黄金大米 SGR1 的种植和收获。颜色、类胡萝卜素含量的指标，无疑是非常令人鼓舞的。大家拍摄了很多照片。尽管先正达公司决定停止他们在黄金大米方面的商业利益，但是先正达公司仍为了发明家的人道主义目的支付了田间试验费用。

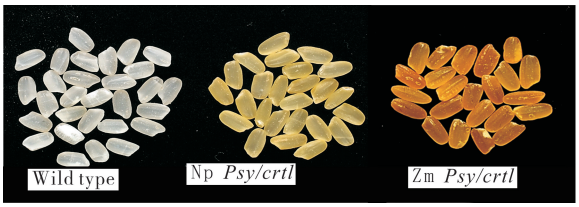

图6 野生类型与T-DNA区含有来自水仙花的*psy*基因(Np)(2000年Ye等已在一代黄金大米中证实)或是玉米来源的*psy*基因(Zm)的转基因水稻米粒,由于类胡萝卜素含量不同显示出不同的颜色

Fig. 6 Wild type and transgenic rice grains containing T-DNA from daffodil *psy* (Np) (as in the proof of concept Golden Rice, Ye et al, 2000) or maize *psy* (Zm) showing altered colour due to carotenoid accumulation (From Paine et al 2005)

图7 2004年美国田间种植的黄金大米SGR1

Fig. 7 US field grown Golden Rice SGR1

2004年6月23日先正达公司宣布:“在植物科学方面,我们集中精力在北卡罗来纳州的先正达生物技术公司(SBI)开展研究和拓展活动,用一个更灵活的形式来汇集更多所需的技术……与此同时,在Jealott's Hill大约会裁掉130个职位,在SBI将会裁掉大约45个研究职位,但是这些将在植物科学发展中由计划内增长来弥补”。

2004年10月14日,为了纪念10月16日的世界粮食日,先正达公司宣布向美国证券交易委员会[20]捐赠“黄金大米种子和株系”(如SGR1),“含有更高含量的β-胡萝卜素的新株系以及相关技术、权利和研究”(如SGR2)给“黄金大米人道主义委员会”。在同一份通告中,先正达公司声明“公司在黄金大米项目不涉及任何商业利益”。

具有生物安全高度预防原则的卡塔赫纳协议书在2003年开始执行。在2005年由于2个不同的Bt玉米的转化事件,未注册的Bt-10和已注册Bt-11在研究链中被混淆,先正达公司也因此陷入了一个国际监管的危机之中。这两种事件在运往国外的商业化供应的玉米中均有发现。反对转基因的组织将该事件作为了一个很好的攻击资本,而这个错误让先正达公司在经济上和名誉上均付出了代价。

2005年的《Nature Biotechnology》上详细描述了SGR2黄金大米转化事件,这让先正达公司支持的发明家的人道主义工程梦想离实现更进了一步:“与先正达公司支持的黄金大米人道主义项目一致,黄金大米2代转化事件在一定许可条件下将被贡献出来用于进一步的研究和开发。这些许可条件应符合黄金大米人道主义委员会战略方向并且要遵守所有的法规。请在初审时直接与Adrian Dubock联系(附上其在先正达公司的Email地址)……”

2005年,一个SGR2转化事件在路易斯安那州开展田间试验,这一次试验费用是由Beyer博士的研究经费支付。

在Bt-10丑闻前,先正达在2004年用一个简单的材料转让协议允许多个SGR1转化事件的生物材料被送往多个国家的若干不同的黄金大米网络合作机构,同时协议中也明确提出材料受人道主义委员会战略管理条例管制,并遵守黄金大米人道主义的条款。然而,当Bt-10和Bt-11事件发生以后,先正达公司产品管理经理很是担心SGR2的管理,因而也更加小心地管理着未注册的黄金大米转化事件以避免潜在的“可能发生的偶然性”。

黄金水稻的有关研究转移到了美国北卡罗莱纳的三角研究园区内的先正达生物技术有限公司(SBI),这使已在SGR2上取得突破性进展的Jealott希尔先正达团队丢掉了工作。在先正达公司停止黄金大米工程商业利益之前,SBI已经将性状整合到在美国常见的商业化*javanica*水稻品种中。

SBI科学家们和管理专家们根据管理标准鉴定出了13个SGR2转化事件。由于考虑到存在的偶然性,所以专家们建议,其中的一个转化事件必须经由SBI选择并提交给人道主义计划。人道主义委员会认为应该在亚洲种质资源中选择符合亚洲国家种植条件的,因为在亚洲维生素A缺乏症是主要问题。通过讨论决定,13个转化事件中的6个转化事件将提供给亚洲的2个水稻研究机构——国际水稻研究所(IRRI)和印度农业研究所(IARI)。这两个机构均通过了先正达公司制定的审核标准,这保证他们能够有效地管理项目中转基因作物的材料。按照和SGR1同样的材料转让协议条款,SGR2材料

提供给了 IRRI 和 IARI 。

4.2　2006－2014 年

在印度和菲律宾关于 SGR2 的计划与其他国家的 SGR1 计划一样，是将黄金大米的基因性状转入到当地重要的且规模化种植的籼稻品种中，从而培育一个含有营养特性并适合当地种植的水稻，同时适用于与其他国家的水稻品种杂交的亲本体系。根据研究项目收集的数据，人道主义委员会打算尽快选择一个主要的转化事件并使之转入到需要这种特性的地方水稻品种中，并在当地注册。这也符合卡塔赫纳生物安全议定书，利于共同承担获得监管数据所需的花费，同时减少意外事件发生的可能性，这也符合 2000 年 8 月第一次委员会会议以来人道主义委员会的战略。在亚洲，对黄金大米无需进行其他的基因工程方面的操作了，只需要进行一些常规育种。

在印度，根据卡塔赫纳协议而制定的当地法规最终导致为了开展转基因作物研究需要建一个非常昂贵的建筑，人称“人工气候室”。授权人员进入“人工气候室”需要通过一个空气锁。在人工气候室的所有植物都生长在人工环境下。令人遗憾的是这样会影响植物的表型，因此，只有遗传标记可以用来跟踪性状基因的稳定性，育种家无法使用在观察和选择表型方面的常规技巧。

在菲律宾，基于卡塔赫纳协议的规定允许使用现代温室（后来在印度也获得批准），使得育种者能够更好通过表型观察进行筛选。

这样的育种过程必然是缓慢的，每次回交对于每个地区的每个品种都需要一个生长周期。这样做的目的是得到这种特性的纯合群体，与背景材料唯一的不同只是引入了该营养性状。

为了协助育种工作，国际水稻研究所请求从先正达公司获得转化事件的有关分子数据。SBI 在 2006 年提供了数据，也仅仅是给了 IRRI。先正达公司没有提供数据，甚至没有给 Beyer 博士，更别说给其他的研究机构了。

在人道主义委员会能够选择主导的转化事件之前，研究者获得了在 4 种不同的遗传背景下不同转化事件农艺性状的数据，以及关于储藏的抛光后的黄金大米中 β-胡萝卜素积累情况的数据。作为转化事件选择的一个标准，计算在黄金大米中需要多少 β-胡萝卜素是必要的。更有必要的了解在黄金大米中的 β-胡萝卜素转化成人体循环的维生素 A 的效率。

由于各种原因（没有动物模型能模拟人类吸收和转化 β-胡萝卜素过程），营养学家认为动物模型对人体试验来说并没有太多帮助。对于维生素 A 缺乏的研究，潜在的首要有效研究目标人群是儿童，但是工业化国家的儿童没有遭受维生素 A 缺乏症之苦，因而之前提到的工作都是计划针对中国儿童的，临床研究人员来自中国和美国。与先前在美国成年人中的研究一样，对孩子们的研究采用了相同的方法，但是由于血液量较少，存在无法产生足够氘标记的 β-胡萝卜素黄金大米的问题。SGR1 的表达水平表明一个儿童一餐所食用的黄金大米的量不能达到预期目的。SGR2 总的类胡萝卜素的表达水平及其中 β-胡萝卜素（血液中生物维生素 A 循环的最重要的有益形式）占 95%，均预示该事件的最终成功。然而，由于氘（重水）的高昂的花费，Mike Grusak 博士和他的团队在贝勒医学院用一个小水培生长箱标记 SGR2，其中冷凝液可以被回收利用。这个团队在水稻水培方面并没有太多经验，因而在获得充足的黄金大米前的 2 轮珍贵的水稻材料都被蚜虫和螨虫吃光了。这使得对中国儿童的研究试验一直拖到了 2008 年。

在国际水稻研究所召开的第 10 次人道主义委员会会议之后，新的执行总监 Robert Zeigler 博士说，他希望接替副执行总监 Ren Wang 和 Willy Padolina 博士（他们分别分管研究和合作）并进入委员会。他的请求很快被接受了。

国际水稻研究所的项目经理和黄金大米网络的协调员 Gerard Barry 博士，在 2003 年 11 月初加入 IRRI 并开始工作。直到 2013 年 12 月离开 IRRI，他一直是委员会的高级委员，弗莱堡的项目经理 Jorge Mayer 博士也是一样（他在 2008 年由于家庭原因离开了弗莱堡）。Meyer 博士目前作为一名志愿者和黄金大米协会的好友，依然管理着黄金大米项目在澳大利亚的网站 www. goldenrice. org。

2006 年 11 月 16 日到 18 日在印度德里召开了第 12 次人道主义委员会，大会建议：为了人道主义工程，公共部门的合作伙伴应该更能接受将 SGR1 和 SGR2 中 的“S”（标志着来源于先正达的转化事件的产品）去掉。该提议通过后，自此之后只用 GR1 和 GR2 来标识黄金大米材料。

2008 年 IRRI 在菲律宾的洛斯巴洛斯开展了黄

金大米的第一个可控的田间试验。种植条件包括与其他水稻品种的物理隔离带:周围的玉米种植带避免花粉漂移,以及周围的高铁丝网。这些条件是当地对于种植GMO作物所提出的要求,也遵守了卡塔赫纳生物安全协议。在菲律宾的稻田还刚好在一场强大的飓风之前收割了材料,躲过了飓风的肆虐。

图8 黄金大米(内部区域)与其他作物的物理距离、利用高杆植物玉米和金属栅栏屏蔽花粉传播,所有的条件符合由卡塔赫纳议定书衍生而来的国家转基因作物管理条例

Fig. 8 Note the physical distance of the (inner area) Golden Rice from other rice, the maize pollen trap and the metal fence, all required by Cartagena Protocol derived national GMO-crop regulations

相比之下,2004年和2005年美国(它不是卡塔赫纳协议签署国)的黄金大米试验田周围只有几行非转基因的水稻用作花粉漂移的屏障,并没有任何形式的围墙。

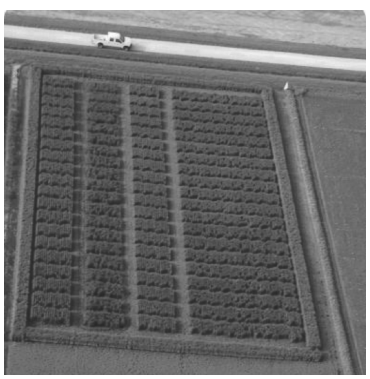

图9 与之相反,美国2004年和2005年的黄金大米田间试验,没有特别的和昂贵的措施,美国不是卡塔赫纳议定书的签署国,就像这样,水稻只有当生长在大田条件下才会展现其真正的表型

Fig. 9 Conversely, in USA field trials of Golden Rice in 2004 (illustrated) and 2005, no extreme and expensive measures were required, USA is not a signatory to the Cartagena Protocol, and rice exhibits its true phenotype only when grown in open field

2009年3月18日和19日,黄金大米人道主义委员会为了选择主导的转化事件和共商接下来的被许可方会议召开了第14届"分水岭"会议。会议开始的前一天,Guangwen Tang博士提交了被美国《临床营养学》杂志接收的文章,该文是关于在美国成年人中开展的人体中黄金大米的维生素A利用率的研究。黄金大米中的β-胡萝卜素转化成视黄醇的比例被证明是3.8∶1,并且这篇文章表明来源于黄金大米的β-胡萝卜素在人体内能够有效地转化成维生素A。这篇文章后来在2009年见刊[22]。

根据管理转基因作物的相关规定,印度关于黄金大米的数据均来自人工气候室,然而由于在人工环境下对作物的表型观察来说是不利的,因此,在印度没有获得相应的数据。因此,在3月召开的委员会会议上只考虑了来自菲律宾的国际水稻研究所的数据。3个来自GR1的转化事件(146、309和652)和6个来自GR2的转化事件(W、G、R、E、L和T)分别在4种目标籼稻品种(IR64、IR36、BR29和Rc82)做了遗传育种,来自这些材料的相关数据被用于分析。国际水稻研究所的水稻育种专家Parminder Virk博士主管这个项目,提供了全面系统的数据,包括水稻育种常用的10个农艺性状,还包括类胡萝卜素含量及其随时间的降解情况。所有数据来自生长在现代温室中的水稻,因为根据条例,转基因作物是不允许在开放的田间种植的。孟加拉国水稻育种家Alamgir Hossain和Partha Biswas博士熟练的技能和对工作的尽职尽责得到了一致的认可。同时,除Virk博士外的另外12个来自国际水稻研究所的员工也参与了这项工作。

这是一个非常庞大、复杂和昂贵的研究计划。在研究中,筛选转化种子时不影响种子萌发的新颖系统被开发出来,同时研究也发现仅仅使用分子标记来进行营养性状的选择是不够的。和所有的作物一样,类胡萝卜素含量在收割后会迅速降低,但是在水稻中收割后2个月内,其降低速率相对最低。

尽管人道主义委员会的营养学家Rob Russell博士没能出席委员会会议,不过Beyer教授在事前就已经向Russell博士完整地介绍了相关研究结果。因此,他在会上向委员会报告了人体需要多少类胡萝卜素来改善个体维生素A状况的计算结果。推荐的维生素A日摄取量包括足以维持对健康个体的3个月的肝脏储存。当然,其实肝脏储存对于克服维生素A缺乏来说是不必要的。所有的计算

(以及后续的育种决策)均用存储 2 个月后的种子中存留的 β-胡萝卜素含量计算。计算中还假设在烹饪中类胡萝卜素有 20%的损失量,而 Tang 博士在会议前的礼拜日提交的数据称只有 6%的损失量。因为有许多不同的方法来烹饪米饭,例如有时所有的水被大米完全吸收而有时煮饭时又用了过量的水最后又被倒掉,所以这种较保守的计算被认为是合理的。Russell 博士的建议是,儿童尤其是有轻微或严重的维生素 A 缺乏症的儿童比成年人更适合用来研究从黄金大米中的 β-胡萝卜素到视黄醇(维生素 A 循环的一种重要形式)的生物转化效率。

在讨论中,来自印度政府生物技术委员会的成员 S R Rao 博士,由于没有来自印度的相关研究数据,所以他最初并不完全支持接受该主导转化事件的决定。他还询问有没有其他可用的分子数据可用来支持该决策。可惜当时并没有现成的数据(尽管国际水稻研究所其实在 2006 年就有相关的数据,但在当时似乎大家都忘记了)。

因为没有详细的分子数据和在印度种植材料的农艺性状数据,委员会经过慎重考虑和讨论还是接受由国际水稻研究所推荐一个主推黄金大米转化事件。这个建议是根据国际水稻研究所的数据、β-胡萝卜素的生物转化比率、饮食中大米食用量的考虑和贮存 2 个月后的黄金大米在烹煮后的 β-胡萝卜素的含量等方面的综合考虑而提出的。分析证明 GR1 转化事件的大米均不能满足从摄入的黄金大米中获得所需的 β-胡萝卜素,但 GR2 转化事件的大米可以。根据数据,大家一致认为 GR2G 材料将会是一个主导转化事件,如果需要的话将 GR2R 作为备用材料。计划是:此主导事件将会被送给所有的黄金大米使用,被许可方用来进一步将目的基因转入到适应本地的水稻品种中,备用转化事件将仅仅由国际水稻研究所保存。基于成本和资源的考虑,以及为了减少由于卡塔赫纳协议引发的潜在的偶然问题,在更多的国家培育更多品种的培育项目没能启动。

在黄金大米网络会议达成一致意见后的 1 个小时内,该计划就启动了。在我们黄金大米人道主义委员会会议中,在这么紧凑的时间内就达成协议是非常必要的,因为委员会从来没有任何资金支持,所以在时间运筹帷幄必须是非常有效率的。整个组织团体的会议在国际水稻研究所的黄金大米网络协调员 Barry 博士的管理下良好地运行着。整个团体的代表来自孟加拉国、印度、印度尼西亚、菲律宾和越南,他们是水稻研究机构中的科学家以及政府负责的高级管理人员。

委员会的主席 Potrykus 教授(也是黄金大米专利的持有人)在委员会上推荐了的主导转化事件 GR2R,并且简单讲述了推荐的原因。在会上,就放弃之前的转化事件的计划展开了讨论。由于是在使用了公共经费的情况下,因此,该议题对任何一名研究人员来说都是一个敏感的话题,但是从产品的管理因素考虑(基于卡塔赫纳协议)还是必需的。同时许多国家提出了他们的育种计划。会议圆满结束。

在 2009 年 12 月 1 日,由于需要紧急商议,而我们又来不及筹备委员会会议,我发了一份电子邮件给人道主义委员会。

"亲爱的同事们:

以下信息已得到我们的主席 Ingo Potrykus 批准。

附件中是今年 3 月在国际水稻研究所举行的人道主义委员会的部分记录草稿。这些草稿涉及到决定推进主导转化事件的会议内容。你可能还记得那是 GR2G 材料。

下面是来源于 Peter Beyer、Gerard Barry 、我自己以及 Mike Grusak (黄金水稻人类研究文章的共同作者,以及 PVMRC 计划的参加人)和 Hector Quemada(来自丹佛斯中心,支持黄金大米的管理学专家,盖茨基金项目主持人)参加的最近在阿鲁沙召开的比尔和梅林达·盖茨全球健康挑战基金会议的摘录。

"就 2009 年 3 月由人道主义委员会筛选的主导转化事件 GR2G 所遇到的问题进行了有关讨论,包括插入序列是不完整的(由国际水稻研究所在 2009 年 3 月下旬发现)。这将影响到启动子的组织特异性表达(由 Peter Beyer 调研,并在 9 月发现其实并不是这种情况),但即使不影响启动子组织特异性,序列的删除也会导致监管问题,并耽搁审核的进程。

Mike Grusak 坚持认为没有理由怀疑来源于不同的转化事件的 β-胡萝卜素生物转化为视黄醇的效率会有任何差异。

据回忆,人道主义委员会上表明从 β-胡萝卜素积累角度说,备用材料 GR2R 比被选为主导转化事件的 GR2G 农艺性状表现更好。GR2G 被选择为主导转化事件是因为它已经被用于人体的生物转化试验。

在场的人一致同意，国际水稻研究所向黄金大米人道主义委员会提出的非正式建议。建议的内容是更换主导转化事件 GR2R 并将 GR2E 备用转化事件以及丢弃 GR2G 转化事件。

不同于先正达公司，根据法律规则，ACD 认为该决定还是需要人道主义委员会最终决议，并且 ACD 将会和我、Potrykus 和人道主义委员会共同商讨”。

行动 1：GB 提供给 ACD 所涉及到的有关证据的一个总结(已完成)。

行动 2：ACD 确保让人道主义委员会通过将 GR2R 作为主导转化事件材料这一决定，并记录在案。

Barry 博士提供的数据是：

·GR2 转化事件中 G、R 和 E 全部测序

—插入序列与原始的转化载体 pSYN12124 序列是完全相同的，没有突变

—除了在 G 转化事件中 crtI 的启动子区域有一段约 400 bp 的缺失

·这个缺失将需要额外的解释和研究来分析这种意外特性的出现

·在插入区段向外的两端的 1 000 bp 以上的序列均已测序

—G 事件插入在外显子中，R 事件插入在内含子中，E 事件插入在一段间隔序列

·所有序列和数据均由人道主义委员会，生物安全资源网络和食物过敏研究与资源计划审查过(除了上述问题确实没有其他问题)

(显而易见的是，大部分的决策受监管系统控制，由成员国根据卡塔赫纳议定书进行改善。除了国际水稻研究所提供的总结性幻灯片，尽管大多数成员接受过这种训练，委员会并没有以任何有效的方式来审查所有的序列信息，也不清楚哪些人或是在哪种监管水平下能够做到这一点)。委员会一致同意更改主要事件转化事件为 GR2R。

2010 年 7 月在巴塞尔协议的公司总部召开了一次会议，国际水稻研究所的总干事、国际水稻研究所网络协调员和先正达公司参加了会议，发明家和作者均没有被邀请参加(尽管其实他们 3 人都住附近)。就在 2010 年 8 月 26 日和 27 日在新加坡召开第 15 次人道主义委员会会议前，国际水稻研究所的总干事和黄金大米网络的网络协调员，会见了发明家和作者，并对 2010 年 7 月会议的一些后期协议草案的内容做了解释。其中一份文件是材料转让协议，此协议比 2009 年 3 月已同意的协议在形式上更加复杂，为了应用的目的将主导转化事件 GR2 联系到黄金大米的许可证上。

目前还不清楚是什么样的想法或哪个组织促使了 2010 年 7 月在瑞士召开的会议。在 2010 年后期 Beyer 教授被比尔和梅林达·盖茨基金会告知，他在 2005 年获得的由该基金会健康部门竞争项目资助将在 2010 年终止且不会再续，该项目是为开发黄金水稻的衍生产品(即改良第二代黄金水稻品种)。不过，基金会打算提供国际水稻研究所一个资助用于黄金大米的培育，为了保证国际水稻研究所在其以外的地方进行黄金大米的管理。

在 CGIAR 研究所召开黄金大米研讨会的 1 天后，第 16 届黄金大米人道主义委员会会议于 2011 年 11 月 13 日在华盛顿的国际粮食政策研究所(IFPRI)召开，在会上国际水稻研究所通报了盖茨基金即将资助黄金大米的好消息，这件事当然是深受欢迎。

2011 年 11 月 3 日，我在巴西圣保罗 the Conselho de Informacies Sobre Biotechnolgia 第 10 届会议上接受了采访。在长长的采访中我提到：“截至 2011 年 10 月的今天，自 2009 年 3 月主导转化事件确定已超过两年半的时间，筛选的黄金大米种子仅仅供应给印度和菲律宾 2 个国家的研究所。其实多个水稻育种项目可以在多个国家进行，发明家和其他国家黄金大米许可方的公共机构对如此缓慢的进展感到很失望。所有的许可方已经拥有黄金大米性状的法律所有权。他们需要生物材料。用同样的生物材料，甚至在不同的水稻品种上运用相同的遗传转化，这些都被监管部门严格监管着。”

Tang 博士在 2003 年 4 月首次提出的关于中国儿童的研究最终在 2012 年 8 月发表[4]。文中表明“黄金大米中 β-胡萝卜素与食用油中的 β-胡萝卜素在提供给儿童维生素 A 方面一样起作用，且比菠菜的效果好。一碗 100～150 g 煮熟的黄金大米(干质量 50 g)可以给 6～8 岁的中国儿童提供推荐的营养摄入维生素 A 的 60%。”“总的来说，黄金大米中 β-胡萝卜素生物转化成维生素 A 较高的生物转化效率显示黄金水稻可以用来作为维生素 A 的来源。在以大米为主食的人群中克服维生素 A 缺乏方面，黄金大米能够与维生素 A 胶囊、鸡蛋或者牛奶来源的维生素 A 来源一样有用。植物性食物中的维生

素 A 等效性认识给在维生素 A 普遍缺乏的地区设计利用食物来提高维生素 A 吸收的营养方案提供了科学依据。”

22 d 后的 2012 年 8 月 30 日，绿色和平组织发布了一份新闻稿，谴责使用转基因作物黄金大米将中国儿童作为美国科学研究的“小老鼠”。实际上，Tang 博士和其他几位参与这项研究的临床医生都是在中国出生或者在中国居住的。Tang 博士有 25 年相关研究的经验，而且他的同事之前也用黄金大米在美国成年人[22]和用其他非转基因作物来源的 β-胡萝卜素[23]在中国儿童中做过类似研究，但是只有 Tang 博士的 2012 年的关于黄金大米的研究受到了绿色和平组织的批评。

正如前文所提到的，在十多年前的 2001 年，绿色和平组织就发布了一份新闻稿称黄金大米在对抗维生素 A 缺乏方面没有那么有效，因为一个成年人每天必须吃 300 g(正常摄入量)未煮过的大米的 12 倍的量，即大约 3.6 kg，才能获得每日所需的维生素 A。2012 年，对于 Tang 博士的结果，绿色和平组织希望能够质疑他发表的结果，但是并没有提出任何实质证据来证实他们的指控。

在 2012 年和 2013 年，国际水稻研究所和菲律宾水稻研究所计划并实施了 5 个地点的多地点田间试验作为黄金大米 GR2G 转化事件监管过程的一部分。田间试验的大概位置在网上发表了。所有地点均是符合规定的，并且遵守转基因作物田间试验的规定，每个地点均被高栅栏围着，同时配备保安日夜巡逻。在 2013 年 8 月 8 日，其中最容易发现的一个田间试验地点被反对转基因作物示威者肆意破坏。当地有关部门觉察到示威者并不是如声明中所称的农民，而是一些来自知名组织的主要煽动者。菲律宾农业部门着手跟踪并起诉了涉案人员[24]。破坏田间试验的这种恶劣行为遭到了科学界强烈谴责[25]。

除了一块试验田被破坏，从其他田间收集来的数据仍是充足的，结果表明：和预期的一样，黄金大米的产量比野生型品种有较好的收益率。对于任何性状，特别是营养强化这一消费相关性状，商业种植者都期待优良的农艺性状。(政府培育一种作物免费提供给营养缺陷群体的项目，项目上有可能会选择不同的标准。)通常商业种植者选择新品种和性状时，主要是针对是否可以增加效益，以及是否易于栽培和收获(两方面都会带来经济效益)。

2013 年 12 月的会议上，国际水稻研究所网络协调员重提了他在 2009 年 12 月幻灯片中总结的问题：“R 转化事件的插入位点在内含子区域”。进一步调查表明分子方面的数据在 2006 年仅仅提供给了国际水稻研究所，数据包括关于内含子的插入位点以及涉及到的内含子是 Aux1(只需要任何在该领域有过涉略的人点 4 次鼠标就可以发现)，从 1999 年就知道它与根的发育相关(P Beyer，个人交流)。

2014 年 5 月 15 日国际水稻研究所在他们的网站发布以下他们关于黄金大米的研究信息：“第一轮的多地点试验用黄金大米最新版本 GR2-R 进行的。2012 到 2013 年在菲律宾不同地点种植的第一轮黄金大米，用来评估这个版本的黄金大米生长状况如何。初步结果喜忧参半。尽管我们的 β-胡萝卜素的目标水平在谷粒中得到提高，但是平均产量却低于当地农民的主栽品种。

试验的一个重要目标是测试这种水稻新品种的农艺性状对于农民来说是否能接受。初步结果表明有必要进行更多的研究，并且也要更多的关注产量的提高。基于这些结果，大家达成了一致意见，“要从只关注 GR2-R 版本的黄金大米推进到包括 GR2-E 在内的其他版本的黄金大米。”……“国际水稻研究所及其许多研究伙伴仍然致力于培育一个高性能的黄金水稻品种造福农民和消费者。黄金大米工程的重要任务是为了致力于改善数百万人遭受微量营养素缺乏的健康问题，要求黄金大米科学研究的每一步和每一方面都要产生好的结果。国际水稻研究所和所有参与组织将在今后黄金大米的研发和推广的过程中继续严格遵守所有其他生物安全和监管协议。”[26]

5　黄金大米的发展历史和轨迹教会了我们什么？

从科学角度来讲，从研究到概念验证，从优化技术到种子育种进而得到一种稳定的作物，一直是一个巨大的挑战。对于一个新领域——利用生物强化作物提高微量元素含量保障食品安全需要：(1)调控新的生物合成途径；(2)建立一个针对亚洲水稻品种转化方法。对于水稻这样一个对于经济、政治和宗教都重要的作物，对于这个跨时区、跨文化、跨农业、营养学和社会学等不同领域的国际项目特别是这样。

随着 Potrykus 和 Beyer 团队最初研究的成功,一个为了不同目标共同努力的创新性公私伙伴合作关系形成了:对于公众而言——在发展中国家展开非营利的人道主义运用,对于公司而言——在欧洲和北美开展对“功能性食物”商业性开发。

由于所有参与的人都热情高涨并且相互间有良好的交流,因而国际合作迅速建立。最初,来自企业的日本、新西兰和英国以及之后的美国农业科学家在黄金水稻研发上的速度是令人钦佩的。来自美国和中国的营养学家和临床科学家们看到了项目的可能性并希望了解其潜力。经费来自于私人和公共部门、政府和慈善团体。国家级以及国际性的水稻研究所带着满腔的热情参与了这项项目,他们分享了在适合当地的优良水稻品种中改良黄金大米的技术和资源。所涉及到的人都明白达到目标的重要性:减轻由于不良饮食患维生素 A 缺乏症的成千上万的人的痛苦。所涉及到的人都明白,贫穷才是根本问题,我们需要通过其他方法解决贫困本身,虽然尽管黄金大米不能治愈贫困,它还是可以帮助人们在贫困中生存下去从而更好地面对他们的机遇。

从 2000 年 8 月的第一次黄金大米人道主义委员会会议以及其后的每个阶段,研发都是在转基因作物的严格监管下进行的。之后,各个国家的管理部门依据具高度预防性原则的卡塔赫纳协议[17]进一步完善了管理条例,对于工业化国家的投资者而言,其商业上的吸引力大大降低。2004 年先正达公司申明放弃其在黄金大米中的商业利益[20]。其实早在 2001 年初完成黄金大米并交给国际水稻研究所时,国际合作者之间分享材料并且共同进行繁育的速度还是较快的,这种交接速度可以和布劳格在 20 世纪 60 年代将小麦种子品种交给国际水稻研究所时的速度相媲美,但是现在这种相互间分享的速度由于受卡塔赫纳协议的限制而越来越缓慢了。

政治激进主义打着关心健康和环境的幌子,利用对转基因作物技术的怀疑给许多不满全球化的积极分子提供弹药。黄金大米人道主义项目单纯的美好愿望对那些激进分子来说是一场他们必须要赢的战斗,而这只是为了意识形态上的胜利[5]。随着争论愈演愈烈,许多机构的参与者更加惧怕“潜在的责任问题”,助长了削减分享研究材料的意愿并且进一步阻碍了合作研究和交流。大多数国际组织悄悄地避开任何援助资金甚至避免任何参与转基因作物(即使是已有明显潜力的黄金大米)有关的话题。

卡塔赫纳议定书带来的影响以及它被许多签署国用做监管的基础是不幸的:“预防原则对于国家政策的制定起着阻碍的作用。它要不就是太显而易见以致变得多余,或者它是如此的模糊以致毫无意义。但是最常见的应用是——‘当一个行为有可能危害环境和人类健康,那么即使一些科学的因果关系还不完全明确,也必须要采取预防措施。’这对于那些想要终止任何他们不喜欢的新科学的发展的人是一个百试不爽的利器。”[27]

卡塔赫纳议定书中有关管理转基因植物研发中最大的反科学阴谋是其阻止了育种者在种植的早期通过表型观察筛选适合的转基因植株。这一制约,无论从环境角度还是人类风险管理角度都是不必要的,却使得将有益性状转入至好的品种中这一育种进程延迟了很多年,并且非常显著地增加了育种的成本和复杂性。

对于所有的作物育种,包括 GMO 作物,其过程必须需要作物育种者的传统技能和在开放式大田中选择植物生长表型。只有在田间种植条件,面临各种生物和非生物胁迫,才方便育种者选择有用的植株丢弃没用的植株。

6 结 论

2004 年,联合国儿童基金会和微量营养素倡导团体在瑞士举行的达沃斯全球经济论坛上发表了一份报告:维生素和矿物质缺乏现状[28]。众所周知我们正在处理一个全球非常重要的却很少人意识到的问题:维生素和矿物质缺乏会影响 20 亿人的健康、智力和国家的经济前景。报告指出,早在 2002 年 5 月联合国大会就曾呼吁到 2010 年消除维生素 A 缺乏症。

不推荐给 6 个月以下的孩子补充维生素 A,而且很小的孩子不会吃固态的食物。可是这些孩子却最容易患上维生素 A 缺乏症:2011 年新生儿死亡人数占 5 岁以下孩子死亡总数的 43%,比起 1990 年增长了 36%[29]。因为母乳能够帮助缓和维生素 A 缺乏症,所以母亲自身不能缺乏维生素 A,体内应有足够的维生素 A 的储存。黄金大米中的 β-胡萝卜素已经被证实有很好的生物利用率[4,22]并且“可以和维生素 A 胶囊、鸡蛋或者牛奶来源的维生素 A 前体在克服维生素 A 缺乏方面一样有帮助。”[4]

对世界上一半以水稻为主食的人群来说,黄金

大米的利用有可能在实现联合国在 20 世纪 90 年代初期制定的目标(尽管 25 年后仍没有实现)方面起着重要作用。

图 10　背景中是黄金大米植株，在培养皿中大约有 100 g 抛光后的黄金大米，数据表明大约每天食用 40 g 煮熟的黄金大米将能预防因维生素 A 缺乏而导致的失明或死亡

Fig. 10　Golden Rice plants in the background, and around 100 g of polished Golden Rice grains in the Petri dish (The data suggests that about 40 grams of Golden Rice cooked and consumed daily will safely prevent blindness or death from vitamin A deficiency)

可悲的是，黄金大米的滞后发展使得全社会不得不承受失明和死亡的痛苦，而这归因于各国基于联合国生物多样性公约和卡塔赫纳议定书形成的一系列管理办法，以及人们和研究院所对待这些协议和规则的方式。卡塔赫纳协议所担忧的事情其实在 50 年前就开始引发争论，直到现在这些担忧已经被证明是不值得的。与使用其他技术育种得到的农作物相比，转基因作物不会带来更大的风险。然而，除了直接成本，这些条例加深了人们对一个有用的、良性的作物育种发展的怀疑。正是因为这些原因，《卡塔赫纳生物安全议定书》对转基因作物来说是不合适的，且没有起到什么积极作用。

关于黄金大米，在过去的 10 年里，仅仅在印度反对转基因作物的花费每年就达到了 2 亿美元[30]。2010 年，维生素 A 缺乏比艾滋病、结核病和疟疾害死的儿童更多。每天都会有 6 000 个本可预防的患维生素 A 缺乏症的人死亡，其大部分都是年轻的孩子。

2004 年的联合国儿童基金会和微量营养素倡议团体报告中指出："我们必须将旧思想抛在脑后，用新知识武装自己，行动起来"。

利用黄金大米是一个简单的、可持续地预防维生素 A 缺乏的办法。最初的梦想是没有局限的。联合国的卡塔赫纳议定书就是"旧思想"，我们必须将其抛在脑后，它没有任何益处，反而阻碍了发展。

尽管存在政治阻碍，但只要我们有耐心，积极寻求经费资助和鼓励机构间合作，相信终有一天，发明家们的梦想终会实现。

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The present status of Golden Rice

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Abstract The development of Golden Rice to date has taken longer than anticipated. It has been proven to have the potential to assist in the alleviation of an important public health problem,vitamin A deficiency,affecting millions. Complying with the highly precautionary,and now proven unnecessary,UN Cartagena Protocol for Biosafety has impeded scientific progress and scientific collaboration,particularly by delaying the selection of phenotypes grown in the open field. So far therefore,Golden Rice has not been able to assist in combatting vitamin A deficiency,identified by the UN as an important public health target for 25 years,and which continues to cause preventable deaths and blindness. However,the inventor's original vision of the donation of the technology to assist the resource poor who want to benefit from it remains firm and achievable,subject to continuing philanthropic and public sector funding.

Key words Golden Rice; vitamin A deficiency; Syngenta;IRRI; Cartagena Protocol for Biosafety; nutrition; public health; rice; UNICEF; UN; WHO

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Keywords Golden Rice; vitamin A deficiency; Syngenta; IRRI; Cartagena Protocol for Biosafety; nutrition; public health; rice; UNICEF; UN; WHO

1 Vitamin A deficiency

Vitamin A deficiency (VAD) affects about 19 million pregnant women and 190 million preschool-age children, mostly from Africa and South-East Asia[1]. The deficiency is the leading cause of childhood blindness[2], with about 500 000 cases annually. When untreated about half of these children die. VAD has also become recognised—only recently—as a nutritionally acquired immune deficiency syndrome[3] resulting annually in the death of one to two million, mostly young children and some mothers. Most of those affected by VAD, do not become blind before dying from diseases which are survivable with a functional immune system (R Russell, *pers comm*.). This severe mortality in 2010[4] globally exceeded mortality caused by HIV/AIDS or tuberculosis or malaria[5].

High under-five mortality, and deep poverty are closely correlated with vitamin A deficiency. Under five years mortality in India is, at more than two million annually, worse than in any other country, probably due to restricted dietary variety together with poor population coverage with vitamin A supplements, and little progress has been made since the 1980's in reducing it[3].

Vitamin A deficiency is also a common nutritional problem in China among the urban and rural populations. In 2004, the prevalence of vitamin deficiency among children of 3 to 12 years old was 9.3%: in the urban areas 3.0% and in rural areas 11.2%. The prevalence of marginal vitamin A deficiency was 45% of the whole population:with 29% in urban and 50% in rural areas[6]. A 2006 survey found VAD affecting 12.2% of Chinese children 0-6 years, and severe VAD afflicting 0.5% of the same age group. Chinese children living in the poor western area having a mother with either poor education or of minority ethnicity have a high risk of VAD[7]. Reviewing a decade's data up to 2005, the World Health Organisation in 2009 reported for China little evidence of night-blindness (an early clinical sign of vitamin A deficiency) in pre-school children or mothers and a mild incidence of VAD in pre-school children. Conversely VAD was reported as a severe public health problem for pregnant women in China[8].

Fig. 1 This Indian young woman, blind in her left eye, is nevertheless lucky, most vitamin A deficiency sufferers die as young children

"Although increasing the consumption of vitamin

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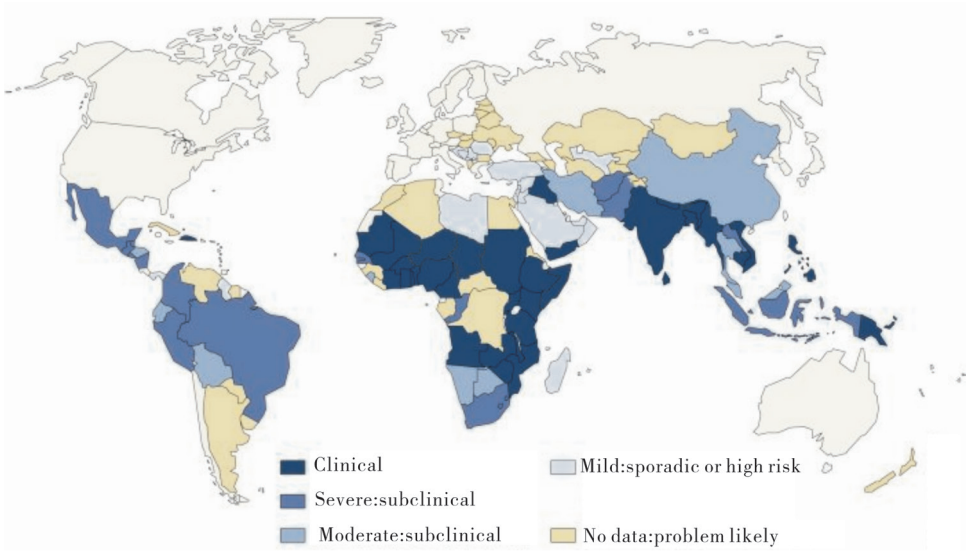

Fig.2 Public health importance of vitamin A deficiency, by country

A-rich foods may seem to be a reasonable solution, in reality, it is much more difficult for pre-school children in poor families to meet the requirements for vitamin A through diet alone. Animal products that are rich in vitamin A, such as liver, eggs, cheese, and butter, are often beyond the reach of poor families. Another critical factor that makes it difficult for pre-school children to meet their dietary requirements for vitamin A through fruit and vegetables alone is that the bioavailability of vitamin A from fruit and vegetables is not high. A young child between ages one year and three would need to eat eight servings of dark green leafy vegetables per day in order to meet the Recommended Dietary Allowance for vitamin A. The problem of the low bioavailability of vitamin A in plant foods has brought the sobering reality of 'the virtual impossibility for most poor, young children to meet their vitamin A requirements through vegetable and fruit intake alone'. The low bioavailability of vitamin A from plant foods explains, in part, the presence of vitamin A deficiency among children living amid ample supplies of dark green leafy vegetables and other plant sources of vitamin A" [3].

White rice, polished so that it can be stored without becoming rancid, contains essentially only carbohydrate, an energy source but not a source of life-essential minerals and vitamins. Vitamin A deficiency is therefore a widespread problem, especially in those large areas of the world where rice is the staple food, and for poor people in these countries 80% or more of their calories may come from rice (H Bouis, *pers comm.*).

2 VAD alleviation-possible, and affordable, but intractable

At the 1990 UN World Summit for Children more than 150 heads of government and senior government officials committed their governments to the virtual elimination of vitamin A deficiency and its consequences by the year 2000[9].

The commitment was strengthened by the 1992 UN International Conference on Nutrition which recognised that the control of vitamin A deficiency is one of the most cost-effective child health and child survival strategies governments can pursue. The conference concluded that all sectors of society should support a combination of strategies to achieve the virtual elimination of vitamin A deficiency. The strategies should include breast-feeding promotion, dietary diversification, vitamin A supplementation, and food fortification[10].

In 2003 UNICEF and the Micronutrient Initiative issued a global progress report "Vitamin and Mineral Deficiency" with the headline 'controlling vitamin and mineral deficiency is an affordable opportunity to improve the lives of two billion people and strengthen the pulse of economic development'. "Probably no other technology available today offers as large an opportunity to improve lives and accelerate development at such low cost" [11].

3 Golden Rice

The term bio-fortification had not been coined at the

time of the UN meetings above. However the research, initiated also in the early 1990s which led to the creation in 1999 of what came to be known as Golden Rice[12], was initiated by the teams of Ingo Potrykus and Peter Beyer in recognition of the same need. Golden Rice is the first purposefully created biofortified crop, designed specifically as an additional intervention for vitamin A deficiency.

Fig.3 The prototype "Golden Rice", described by Ye et al, 2000

Biofortified staple crops, that is crops bred by any method including genetic engineering when necessary, to include enhanced micronutrient—vitamin and or mineral -content or bioavailability are expected to be significantly cheaper, and more sustainable and cost effective in reaching populations than supplementation ('vitamin pills') or fortification (minerals or vitamins added to processed food) to address micronutrient deficiencies[5].

Golden Rice was first widely publicized in 2000 on the front cover of the American (illustrated) and Asian editions of Time magazine.

What progress has been made since, and what is the current status of the project?

Fig.4 Time magazine, September 2000

4 The vision for the Golden Rice project

The vision for the global humanitarian Golden Rice project remains as it was when the creators of Golden Rice started their research: to make available to those resource poor rice consumers in developing countries who wanted it and could benefit from it, a costless source of vitamin A in their staple food rice.

On 5th March 1999, the inventors filed a patent application for the nutritional technology they had invented. Following their ground breaking bio-fortification proof-of-concept success[12], the International Rice Research Institute, in the Philippines (showing an early appreciation of the potential) asked the Rockefeller Foundation to undertake an intellectual property (IP) audit of the technology. The Rockefeller Foundation contracted this task to the International Service for the Acquisition of Agri-biotech Applications (ISAAA), who in turn subcontracted it to a unit at Cornell University run by the Executive Secretary of ISAAA.

The inventors were unimpressed with the attitude of a company individual who contacted them having read about their invention. Contrary to the inventors publicly stated plans to give the technology away free to those that needed it, the manager insisted, that—due to an unrelated previous Material Transfer Agreement which also included one of the technologies used in the Golden Rice research—the nutritional technology was his companies to manage, not the inventors. In their frustration at the manager's intransigence in discussion, the inventors assigned on 20th February 2000 the relevant patents and all their rights to Greenovation, a spinoff biotech company of the University of Freiburg.

At almost the same time Zeneca Agrochemicals (soon to become Syngenta by merger with Novartis) approached the inventors for rights to the technology and were referred to Greenovation. On request, Greenovation promptly granted, on 14th April 2000, exclusive rights to Zeneca, free of charge for humanitarian applications, but royalty bearing for commercial applications.

Zeneca then, on the same day 14th April 2000, granted licenses back to the inventors in order that they could fulfil their commitments to make the technology available, free of charge, to resource poor farmers in developing countries. Through the creation of this public private partnership, the inventors traded commercial rights in the technology to Zeneca, in return for the companies support for the inventor's humanitarian vision. At the time Dr Beyer commented:"Zeneca is the only company worldwide with a long-standing reputation in investigating molecularly carotenoid biosynthesis in plants, therefore Zeneca is our natural partner". Zeneca explained in a press release that "the technology, or transformed rice

seed, will be provided to international and national research organisations, upon request, in developing countries under carefully controlled conditions, who will be assisted in its application to locally available and adapted rice varieties for biosafety and other assessments". When approved by the appropriate national authorities, who will assess safety to man and the environment, the Golden Rice seed can then be multiplied by conventional seed multiplication processes and distributed to resource poor farmers for planting, harvesting, small scale commercial activity (neighbours and local markets) and consumption.

This collaboration will provide Golden Rice and or the relevant technology free of charge to international and national research organisations, who will be licensed to make available rice seed containing the trait to resource poor farmers. Even if rice seed is sold on commercial terms locally, the trait will have to be provided free of charge. Zeneca further stated that "We can also support the necessary biosafety and risk assessment work, transform common public varieties (eg IR64) through Orynova, our Japanese rice affiliate company, with whom we have recently initiated discussions".

When published, the Cornell University IP analysis[13] was unhelpful. On 31th May 2000 Prof Potrykus e-mailed the first author:"Your analysis has led to a frightening picture of the future: how should one be able to achieve freedom to operate for the golden rice if 32 patent holders have to be asked to release their patent rights for the humanitarian project for 78 rice-growing countries". The first part of the Zeneca support to the inventors was to complete a rational IP audit, and determine that actually only a handful of patents may have been infringed by the inventors research, and to agree with the holders of that IP that it would be made available free of charge for the well-defined humanitarian purpose[14-15].

The Zeneca agreement with the inventors mandated that all improvements to the technology, from either party, would be cross licensed. (A few years later Greenovation, trying to raise capital from venture capitalists ('VC') to fund their pharmaceutical biotechnology strategy, were obligated by the VC firm, because of the contentious nature of agricultural biotechnology in Europe, to divest their commercial interests in the pro-vitamin A technology before the VC would advance any capital. Greenovation approached Syngenta for assistance who bought back the rest of the rights from them.)

Through these mechanisms the inventors donated their invention to the resource poor of the world so as to make the nutritional technology available free of charge in public sector rice varieties to those populations which could benefit from it. It is important to emphasise that no one involved with the development of Golden Rice will benefit financially from its adoption[5].

4.1 2000-2005

Following the initial agreements between the inventors and Zeneca, very quickly (and of course coincidentally) followed the merger between Zeneca a UK based agribusiness company and the agribusiness part of Novartis a Swiss based one to form Syngenta, also Swiss based.

The merger was announced to Zeneca staff on 2nd December 1999. In January 2000 Science published the breakthrough of producing beta carotene in otherwise white rice endosperm by the teams of Potrykus and Beyer[12]. The stock market listing of the new company Syngenta in New York, London and Zurich occurred on 13th November 2000, and on 20th January 2001 the Potrykus and Beyer agreement with Zeneca was novated to Syngenta. By April 2001 I had a work permit and was working with Syngenta as Global Head of Mergers & Acquisitions, Ventures and IP Licencing, and was living in Switzerland, fortuitously convenient for contact with Potrykus and Beyer who both lived and worked close by.

The levels of beta-carotene in the inventors proof-of-concept biofortified rice were criticised by Greenpeace as being insignificant for alleviation of vitamin A deficiency in a 2001 press release. Greenpeace, already opposed for 5 years to all genetically modified crops, said in a press release that a breast feeding woman would have to eat 18 kilograms of cooked Golden Rice daily to obtain any benefit. Without knowledge of the bioavailability of the carotenoids in Golden Rice, which was not known at the time, no-one was in a position to make that judgement (which has subsequently been disproved). In February 2001 Charlie Kronick of Greenpeace was reported in the Guardian Newspaper (UK) "Our view is that the billions of pounds that has been spent developing this rice is diverting resources from more sensible ways of tackling VAD"[16]. These were the first of many exaggerations by opponents. In the same Guardian Newspaper report Gordon Conway President of the Rockefeller Foundation joined anti-GMO activist Vandana Shiva in agreeing that "the public relations uses of golden rice have gone too far" [16]. It transpired that US television was showing

advertising images paid for by the US biotech industry implying fields of golden rice were growing in US fields—an embarrassing surprise to those involved in these very early stages of the project in Europe (and of course also misleading).

In any event Syngenta, now keen to optimise the technology for commercial exploitation in 'functional foods' in North America and Europe initiated its research, including a collaborative project between Syngenta's research scientists at Jealott's Hill International Research Station, Bracknell, to the west of London, and Dr Peter Beyer's lab at the University of Freiburg, investigating options for improving on the prototype.

A volunteer Golden Rice Humanitarian Board was also created by invitation to advise the inventors and guide the expected ethical challenges which may arise with the first Board meeting occurring at Zeneca, Fernhurst, UK on 18th August 2000 where a mission statement was agreed. In part it stated:"The Humanitarian Board believes that GoldenRice™ has the potential to be a valuable tool in alleviating vitamin A deficiency in malnourished populations in the developing countries. The Humanitarian Board also believes that GoldenRice™ warrants careful but urgent local work to test it for environmental effects, human safety and benefit. "

One of the first agenda items was to hear the advice of a Zeneca biotechnology regulatory specialist about the molecular characteristics required of a genetically transformed GMO-crop to ensure that it would be able to be registered for use under the regulations to be derived from the very recently (2000) published, but not yet in force (2003) Cartagena Protocol on Biosafety[17]. It was also advised, again with respect to the ramifications of the Cartagena Protocol, and agreed at that first meeting that it would be ideal for only one transformation event of Golden Rice to be developed everywhere, independent of the rice variety into which it was introduced by conventional rice breeding.

At the same time as formation of the Golden Rice Humanitarian Board, and following the agreement between Syngenta and the inventors[5], a network of public sector rice research institutions was formed to start the process required to fulfil the inventors vision. The first was the International Rice Research Institute in the Philippines where its then director Dr Ron Cantrell signed the licence agreement with Professor Potrykus with an effective date the same as that between Potrykus and Syngenta: 20th January 2001. By 22th January 2001 samples of Golden Rice had been delivered by the inventors and I, to IRRI. The Golden Rice seed was hand carried to IRRI by the inventors: the Cartagena Protocol had not yet come into force. Dr Cantrell said in a press release of IRRI, the Rockefeller Foundation and Syngenta:"The arrival of these initial samples at IRRI is a very significant step and allows us to finally start on the required testing processes using local rice varieties. IRRI expects to play a major role in the ongoing "Golden Rice" research effort and its eventual introduction to the world's millions of rice farmers and consumers. Others followed including the Philippine Rice Research Institute and similar institutions in Bangladesh, China, India, Indonesia, South Africa and Vietnam".

Following the 5th Humanitarian Board meeting in Beijing on 21th September 2002, the Board asked the licensee network to create 1 000 plus transformation events, based on the proof of concept constructs successfully created by Prof Beyer's team, from which it was hoped an improved transformation event could be selected collaboratively, to be taken forward as the one lead event by all Golden Rice licensees.

In an e-mail dated 24th March 2003, Dr Gurdev Khush, Humanitarian Board member, former rice breeder at IRRI and World Food Prize Laureate, advised "the Humanitarian Board that "regulatory clean" IR64 with only one copy of vitamin A genes does not need further breeding work. It is just like any other variety. However, during field test its yield potential and alteration in any morphological traits should be evaluated".

By the 6th Board meeting in Zurich in April 2003 no new transformation events had been created by the public sector research institution network. Excitement was created however by the announcement at the same Zurich meeting, of progress having been made by Orynova, a joint venture company between a Syngenta subsidiary company, Mogen BV of the Netherlands, and Japan Tobacco in Japan the latter of whom had novel technology for removing the selectable marker which had been used. About 800 SGR1 transformation events had been created in Japan. Ten transformation events with a single locus, good colour and no marker gene had been selected and T2 plants were growing in UK. The best of the events were expressing 13 μg/g total carotenoids, compared with 1.6 μg/g in the proof of concept Golden Rice. EU regulators had already expressed interest, and there was discussion of plans for EU and US field trials in 2004 with a T4. Additionally, about 200 transformation events had been generated in Peter Beyer's lab. Of these in total about

1 000 transformation events, 4 from Syngenta and 2 from Freiburg/ETH/Cu Long Delta Rice Research Institute, Vietnam (by Dr Hoa, in Beyer's Freiburg lab) were selected for field trialling.

Fig. 5 Golden Rice IR64 transformed by Dr Hoa of Cu Long Rice Research Institute, Vietnam, in Prof Beyer's Lab in Freiburg, Germany

In an October 8th 2003 e-mail to the Golden Rice Network, Prof Potrykus wrote: Golden Rice field trials are "at an advanced planning stage …in Spain and USA and we would also like to involve also Bangladesh, India, the Philippines and Vietnam…these trials can generate data on agronomic performance and trait stability, and generate seed increases to be harvested during 2004, the UN Year of Rice announced by [UN Secretary General] Kofi Annan earlier this year… This is a tremendous challenge and needs to involve those members of the network who have the necessary expertise… We will also benefit from your interaction with the local regulatory authorities to assist the process as fast as possible within guidelines… Golden Rice IR64 seeds are already in Vietnam and India. Before the January [planning] meeting we will also endeavour to arrange transfer of the seed incorporating the new Syngenta events with higher level of expression for inclusion in the same trials programme, and the Rice IR64 to Bangladesh and the Philippines… Planning and funding for the human feeding trial has been achieved in the US by…Humanitarian Board member Dr Robert Russell and his collaborators. Permission, including ethical clearance, is currently being progressed for the trial to be conducted in China, planned to commence also in 2004. "

The e-mail also announced "encouraging developments in the basic science, which may eventually result in an improved "Golden Rice 2" containing further increased β-carotene levels. "

By 3rd November 2003, following this author's proposal of the need from Syngenta, IRRI had recruited a Golden Rice network coordinator, Dr Gerard Barry, funded by USAID and agreement was also in place to recruit a University of Freiburg Project Manager, to assist the Board, with Syngenta Foundation funding, expected to be in place by early 2004. (Dr Jorge Mayer filled this latter post 2004-2008, when he returned to Australia for family reasons, and then this author 2008-2010, following retirement from Syngenta).

A large planning meeting for field testing initiated by Prof Potrykus and Dr Swaminathan, with Golden Rice network participants took place in Delhi on 15th December 2003. Dr Barry took excellent minutes.

Within Syngenta, biotechnology management in 2003 and 2004 was facing tough choices. Following the creation of Syngenta, the portfolio of both legacy companies' biotechnology projects was too large for all to be properly funded. More progress could be made on those with more significant commercial prospects if more resources could be deployed, and to release those resources some of the least valuable projects needed to be dropped.

Benedikt Haerlin Greenpeace's European anti-GMO campaign director had stated in early 2001 that Golden Rice posed a moral challenge to Greenpeace which would therefore not attack field trials of Golden Rice in the Philippines. Nevertheless "The debate on the virtues and perils of biotechnology in the production of transgenic crops …has become quite contentious. . . in recent years… to the extent that it is now delaying and/or preventing the adoption of this important technology in addressing critical and urgent problems of food security and the environment"[18]. This was especially so in most of Europe. Syngenta decided, without at this stage any public announcement, to cease its commercial interest in the development of Golden Rice. It still had its legally binding contractual obligations to support the inventor's humanitarian project, which was also widely popular with Syngenta staff, including a positive role in motivating new recruits to seek to join the company.

The attitude to GMO-crops in other parts of the world was more relaxed. Following the 'approval for cultivation of three Bt-cotton hybrids last year' [Indian] Agriculture Minister Rajnath Singh announced on 18th December 2003 that "A network of projects on transgenics, covering 12 crops is on the anvil. ""The proposed Indian Council of Agricultural Research (ICAR) will cover maize, pigeonpea, chickpea, soybean, cotton, brassica, tomato, Brinjal, banana, papaya, potato and cassava" and focus on a variety of traits. Even in UK on 11th February 2004 " it was agreed between senior cabinet ministers including the foreign secretary, Jack Straw, and the environment secretary, Margaret Beckett that the government would give the green light to the first crop of GM maize in Britain…. The public is unlikely to be receptive. ""Mrs

Beckett said there was no scientific case for an outright ban on the cultivation of GM crops"[19].

In January 2004 Dr Rachel Drake, project leader for Syngenta at Jealott's Hill reported internally within Syngenta variation in carotenoid content between samples of the same SGR1 events analysed at different intervals after harvest, as well as the expected gradual drop in carotenoid content in storage. "The new data underlines the importance of the multi-location field trials in assessing the performance of the trait". By 13th February 2004 she was seeking approval from me to proceed with the internal processes necessary to donate, and archive SGR1 and move on to SGR2. The key meeting of this process occurred at Jealott's Hill on March 31th and 1st April 2004.

By March 2004, 30 high beta-carotene expressing transformation events of the new constructs anticipated in Prof Potrykus' October 8 2003 e-mail had been created by Dr Drake's team. These were already known

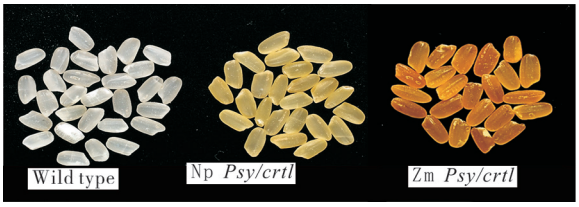

Fig.6 Wild type and transgenic rice grains containing T-DNA from daffodil psy (Np) (as in the proof of concept Golden Rice, Ye et al, 2000) or maize psy (Zm) showing altered colour due to carotenoid accumulation (From Paine et al 2005)

as SGR2 events.

The 8th Golden Rice Humanitarian Board meeting occurred in Louisiana, USA in mid-September 2004, and there was great and continuing excitement to arrive to witness for the first time open field grown and harvested Golden Rice SGR1. The colour, an indication of carotenoid content, was immensely encouraging. Many photographs were taken. Despite Syngenta having decided to cease its commercial interest in Golden Rice, the field trial was paid for by Syngenta to

Fig.7 US field grown Golden Rice SGR1, 2004

support the humanitarian project of the inventors.

On 23th June 2004 an announcement was made in Syngenta:"In Plant Science we are concentrating research and development activities at SBI [Syngenta Biotechnology Inc.] in North Carolina, bringing together the skills required for success in a more flexible organisation…. At Jealott's Hill around 130 positions will be lost. At SBI around 45 research positions will be lost, but these will be offset by planned increases in Plant Science development. "

On 14th October 2004, to mark World Food Day on October 16th, Syngenta announced to the US Securities and Exchange Commission[20] that it was donating "the new Golden Rice seeds and lines"[eg SGR1], including "new lines containing significantly higher levels of beta-carotene as well as the related technology, rights and research" [eg SGR2] "to the Golden Rice Humanitarian Board". In the same announcement Syngenta stated "The company has no commercial interest in the Golden Rice project".

The highly precautionary Cartagena Protocol on Biosafety had come into effect in 2003. In 2005 Syngenta was involved in an international regulatory issue as a result of two different transformation events of Bt-maize Bt-10 (unregistered) and Bt-11 (registered) being mixed in the research chain. Both events were found to be in commercial supplies of maize being shipped internationally. Anti-GMO organisations found this to be good political capital, and the error cost Syngenta financially and reputationally.

The SGR2 Golden Rice transformation events were described in detail in Nature Biotechnology in 2005[21], which also made clear Syngenta's support (legally obligated under its licence agreements with the inventors) for the inventor's humanitarian project:"Consistent with Syngenta's support of the Humanitarian Project for Golden Rice, Golden Rice 2 transgenic events will be donated for further research and development through license under certain conditions. Such conditions including being governed by the strategic direction of the Golden Rice Humanitarian Board and full regulatory compliance. Please direct requests to Adrian Dubock [with Syngenta e-mail address] in the first instance….". One of the SGR2 events was field trialled again in Louisiana in 2005, this time paid for by Dr Beyer's research budget.

Pre the Bt-10 scandal, Syngenta had allowed the physical materials of several SGR1 transformation events to be sent to a number of different Golden Rice network collaborating institutions in several countries using a simple 2004 Material Transfer Agreement making it clear that the materials were under the strategic management of the Humanitarian Board and

subject to the Golden Rice Humanitarian Licenses terms. However, post the Bt-10/Bt-11 embarrassment, when it came to SGR2 Syngenta product stewardship managers were concerned to more carefully manage potential 'adventitious presence' of unregistered Golden Rice transformation events.

The Golden Rice research had transferred to Syngenta Biotechnology Inc. (SBI) at Research Triangle Park, North Carolina, USA and the Jealott's Hill Syngenta team which had made the SGR2 breakthroughs had lost their jobs. At SBI the trait had been incorporated into *javanica* rice varieties, as these are the varieties commonly commercialised in USA, before cessation of Syngenta's commercial interest in the project.

Thirteen SGR2 transformation events had been identified all of which were considered by SBI scientists and regulatory specialists to be ready for and capable of complying with regulatory studies and standards. It was suggested, because of the adventitious presence concern, that one event would be selected by SBI and provided to the humanitarian project. The Humanitarian Board however suggested that it was necessary to select events in Asian germplasm and in Asian conditions, as this was where vitamin A deficiency was principally the problem. Through discussion it was agreed that 6 transformation events of the 13 would be supplied to only two Asian rice research institutions. IRRI and the Indian Agricultural Research Institute, both succumbed to and passed a physical audit by Syngenta of their capability to effectively manage programmes involving GMO-crop materials, which neither had significant previous exposure to. The SGR2 materials were then supplied to IRRI and IARI under Material Transfer Agreements with the same terms as for SGR1.

4.2 2006-2014

The plan in both India and the Philippines for SGR2, as well as other countries for SGR1, was to introgress the Golden Rice nutritional trait into locally important mega-rice varieties of *indica*, to create a breeding parent rice line containing the nutritional trait with which locally adapted and preferred rices could be crossed in each country. From the data accumulated as a result of the research programme the Humanitarian Board intended, as soon as possible, to select one lead transformation event to introgress into all varieties everywhere the trait was required, and to be registered in those territories also. Again this was to fulfil the demands of the Cartagena Protocol on Biosafety, to share the costs of developing the regulatory data package, and to reduce the possibilities of adventitious presence as had been the Humanitarian Boards strategy since the first Board meeting in August 20th. No genetic modification was necessary for Golden Rice in Asia, only conventional breeding.

In India the local regulations in place as a result of the Cartagena Protocol resulted in a very expensive construction known as 'the Phytotron' for GMO-crop research. Entry for authorised personnel was through an air lock. All plant growth in the Phytotron was in artificial environments. Regretfully this affected the plants phenotype, so that only genetic markers could be used to track trait introgression, and the normal seed breeders skills of observation and selection for phenotype could not be employed.

In the Philippines, the regulations (also based on the provisions of the Cartagena Protocol) allowed the use of screen-houses, (later adopted also in India) which allowed better use of seed breeder's skills of phenotyping.

Such breeding necessarily is slow:each backcrossing taking a growing cycle applicable to the variety and the location. The aim was to get to homozygous populations for the trait, where the only difference from the background rice variety was the introduced nutritional trait.

To assist the breeding work, IRRI requested molecular data concerning the transformation events from Syngenta. This was provided in 2006 by SBI solely to IRRI. Syngenta did not provide it, even to Dr Beyer, nor to any other institution.

Before the Humanitarian Board could select the lead event, data concerning the agronomic performance of the different transformation events, in four different rice germplasm backgrounds was generated, and also data about beta-carotene accumulation in stored polished Golden Rice. To calculate how much beta-carotene was needed in the Golden Rice as one of the criteria for event selection, it was necessary to know with what efficiency the beta-carotene in Golden Rice is converted to circulating vitamin A in the human body.

For a variety of reasons nutritionists advised that animal models were not a useful paradigm for such important human estimations. As the principal target for the potentially powerful new intervention for vitamin A deficiency was children, and children in industrialised countries do not suffer from vitamin A deficiency, as previously mentioned work was planned involving children in China and clinical researchers based in China and USA. The same methodology was to be used with the children research, as previously with

adults in USA, but given smaller blood volumes there were problems producing enough deuterium labelled beta carotene Golden Rice. The levels of expression in SGR1 proved insufficient for the quantities of Golden Rice expected to be consumed in a single small meal by a child. SGR2 levels of total carotenoids, and the higher proportion (up to 95%) of beta-carotene (the most important for benefiting changes in circulating vitamin A in the blood) both augured well for eventual success. However, due to the high (US $1.0 million) cost of the deuterium (heavy water) to be used, only a small hydroponic growth chamber could be used in Baylor college of Medicine where labelled SGR2 was produced by Dr Mike Grusak and his team, with condensate being recycled. The team had little experience of growing rice hydroponically. Two crop cycles of this expensive rice were consumed by fungus and then by mites, before sufficient Golden Rice could be produced. This delayed the field phase of the Chinese children research until 2008.

After the 10th Humanitarian Board meeting at IRRI, the new Director General, Dr Robert Zeigler, said that he wanted to join the Board replacing Drs Ren Wang and Willy Padolina, both deputy Director Generals (for research and partnerships respectively). This was immediately accepted within a few minutes of the suggestion. (The IRRI project manager and Golden Rice Network Coordinator, Dr Gerard Barry, who joined IRRI on taking up his position in early November 2003, remained an *ex officio* member of the Board until he left IRRI in December 2013, as did the Freiburg based Project manager Dr Jorge Mayer until he left Freiburg for family reasons in 2008. Dr Meyer still manages the www.goldenrice.org web site from Australia as a volunteer and very good friend of Golden Rice.)

At the 12th Humanitarian Board meeting in Delhi on 16-18 November 2006, it was suggested that for the humanitarian project it would be more acceptable to

Fig.8 Note the physical distance of the (inner area) Golden Rice from other rice, the maize pollen trap and the metal fence, all required by Cartagena Protocol derived national GMO-crop regulations

the public sector partners for the "S" (signifying the Syngenta source of the transformation events) to be dropped from SGR1 and SGR2. This was agreed and henceforth only GR1 and GR2 were used.

In 2008 IRRI planted the first confined field trial of Golden Rice at their location Los Baños, Philippines. The planting conditions included physical isolation from other rice crops, a surrounding belt of maize plants as a pollen trap, and surrounding that a high wire fence these conditions being mandated by the local regulations for GMO-crops developed to comply with the Cartagena Protocol on Biosafety. The field in the Philippines was harvested just in time before a powerful cyclone would have destroyed it a day later.

By contrast, in the 2004 and 2005 Golden Rice field trials in the USA—which is not a Cartagena Protocol signatory—only a surrounding few rows of non-GMO-rice were used as a pollen trap-with no fencing of any kind.

Fig.9 Conversely, in USA field trials of Golden Rice in 2004 (illustrated) and 2005, no extreme and expensive measures were required. USA is not a signatory to the Cartagena Protocol, and rice exhibits its true phenotype only when grown in open field conditions, as here

On 18th and 19th March 2009 the Golden Rice Humanitarian Board assembled for its 14th "watershed" meeting to select the lead event and share the plans with a licensee meeting immediately following.

The day before the meeting started Dr Guangwen Tang provided the manuscript, accepted for publication in the American Journal of Clinical Nutrition, describing the research undertaken with human adults in USA to to determine the vitamin A value of intrinsically labeled dietary Golden Rice in humans. The conversion factor of Golden Rice β-carotene to retinol was demonstrated to be 3.8 to 1 and the paper concluded that the β-carotene derived from Golden Rice is effectively converted to vitamin A in humans. The paper was published later in 2009[22].

Indian Golden Rice data was only available from the phytotron, due to the regulations governing GMO-

crops, and the phenotypes were so adversely affected by the artificial environment that useful data could not be generated.

So at the March Board meeting it was only possible to consider the agronomic data from IRRI in the Philippines. Data from 3 GR1 transformation events (146 309 and 652) and 6 GR2 transformation events(W, G, R, E, L & T) each in 4 target indica rice varieties (IR64, IR36, BR29 and Rc 82) were considered. Dr Parminder Virk, IRRI rice breeder in charge of the programme, presented comprehensive data generated covering ten agronomic measurements used by rice breeders as well as carotenoid content and degradation over time. All data was derived from rice grown in screen houses:open field growth was not permitted under the regulations in place for GMO-crops. The skilled and committed work of Bangladeshi rice breeder Alamgir Hossain and PhD Scholar Partha Biswas, then working at IRRI, were particularly acknowledged, but the team also involved, apart from Dr Virk, 12 other IRRI staff.

This was a very large, complex and expensive research programme. During the research novel systems for selecting transformed seeds without affecting germination had been developed, and it was noted that molecular markers alone were insufficient for nutritional trait selection. Carotenoid content degrades rapidly after harvest (as is common in all crops) but in rice the rate of decrease after 2 months was demonstrated to be minimal.

The Humanitarian Board's nutritionist Dr Rob Russell could not be present at the Board meeting and Professor Beyer who had briefed himself thoroughly with Dr Russell beforehand took the Board through the calculations of how much carotenoid was needed to improve retinol status of individuals. Recommended daily allowances for vitamin A include sufficient to maintain 3 months liver store in healthy individuals. The liver stores are not however necessary to combat vitamin A deficiency. All calculations(and subsequent breeding decisions) used only the retained β-carotene content after 2 months of storage. Calculations also assumed 20% losses of carotenoid through cooking, although only 6% losses had been noted by Dr Tang who sent the data on the Sunday before the meeting. This conservatism was considered sensible as there are many different systems of cooking rice: in some all water is absorbed for instance, and in some excess water is used and discarded. The advice of Dr Russell was that children, and particularly marginally or more severe vitamin A deficient individuals would be expected to demonstrate even more efficient bioconversion than adults of the β-carotene in Golden Rice to retinol the most important form of circulation vitamin A.

During the discussion Board member Dr S R Rao from the Department of Biotechnology, Government of India was initially not fully supportive of taking the lead transformation event decision without considering similar data from the Indian research. He also asked if there was any molecular data available to support the decision making. No such data was forthcoming (although IRRI had received it in 2006, it appeared to have been forgotten).

In the absence of detailed molecular data and Indian agronomic data the Board nevertheless after careful consideration and discussion accepted the IRRI recommendation for a lead Golden Rice transformation event. The recommendation was based on integration of the IRRI data and the bioconversion ratio of the β-carotene, as well as considerations of dietary intake of rice and levels of β-carotene expected after Golden Rice stored for at least two months had been cooked. The analysis demonstrated that none of the GR1 events could provide the required amount of β-carotene in a sufficiently small ingestion of Golden Rice, and that all the GR2 events could. It was agreed, based on the data, that event GR2G would be the Lead Transformation Event, with event GR2R as a back-up event if needed.

The plan was that the Lead Event would be distributed to all Golden Rice licensees for further introgression into locally adapted varieties of rice, and that the back-up event would be retained by IRRI only and progressed in backcrossing stages in parallel with the R event. Cost and resource considerations, as well as concerns to minimise potential adventitious presence problems, due to the Cartagena Protocol's influence prevented more ambitious breeding programmes including more events in more countries.

Only an hour after reaching this decision the Golden Rice Network Meeting was scheduled to start. Such close temporal alignment was always necessary in our Golden Rice Humanitarian Board meetings as the Board has never had any funding, and so time and airfare management had to be very efficient. The network meeting was excellently organised by IRRI's Golden Rice Network coordinator, Dr Barry. Representation from the network came from Bangladesh, India, Indonesia, Philippines and Vietnam with representation from each public sector rice research institution including both the involved scientists as well as the senior administrative function

responsible in the country.

Professor Potrykus, the Chairman of the Board (and the licensee of the Golden Rice technology) presented the decision of the Board that the lead event was GR2R and the reasoning in brief. Plans for destruction of previous transformation events, a sensitive issue for any researchers especially when public money has been used, but necessary for (Cartagena Protocol inspired) product stewardship reasons were discussed, and the countries (knowing in advance that there would be a selected lead event) presented their breeding plans. The meetings closed.

On 1st December 2009 I circulated the following e-mail to the Humanitarian Board, as urgent decisions were required and no physical Board meeting was planned.

"Dear Colleagues,

The following message has been approved by our Chairman, Ingo Potrykus.

I attach part of the Draft Minutes of the Humanitarian Board meeting held at IRRI in March this year. These minutes refer to that part of the meeting which decided on the lead GR event to take forward. You may remember that it was GR2G.

What follows immediately below is an excerpt from a [*Bill & Melinda Gates Grand Challenges in Global Health*] meeting in Arusha recently attended by Peter Beyer; Gerard Barry and myself together with Mike Grusak (one of the co-authors on the GR human studies papers, and part of the PVMRC project) and Hector Quemada (of the Danforth Centre who is a regulatory specialist supporting GR and funded by the Gates Foundation).

"1. There was discussion of the problems encountered with GR2G, the lead event selected at the HumBo in March 2009, relating to the sequencing of the insert, which was found by IRRI, post March 2009, to be incomplete. This may affect the tissue specificity of expression of the promoter, (being investigated by PB, and subsequent to September found not to be the case) but even if this is not the case having deletions will cause regulatory questions which will delay the submissions review.

Mike Grusak confirmed that there was no reason to expect any difference in bioconversion of β-carotene to retinol due to different transformation events being the source of the β-carotene.

It was recalled that at the Humanitarian Board the reserve event GR2R performed better agronomically and from a β-carotene accumulation perspective than the event GR2G selected as the lead. The reason, in these circumstances that GR2G was selected was because it has been used in the human bioconversion trials.

It was unanimously agreed by those present that IRRI's informal recommendation to the Golden Rice Humanitarian Board to change the lead event to GR2R and bring forward GR2E as reserve, and to drop GR2G was full supported.

ACD confirmed that for legal compliance—opposite Syngenta—this decision needs to be a Minuted Decision of the Humanitarian Board, and ACD would arrange this with I Potrykus and the Humanitarian Board.

a)Action: GB to provide ACD a summary of the evidence involved. (Done)

b)Action: ACD to arrange for the Humanitarian Board to endorse the change in lead event to GR2R in a form which satisfies the requirement for a Minuted Decision. "

The data presented by Dr Barry were that:

- ►GR2 events G, R and E sequenced entirely (in the original Kaybonnet)
  —Inserted sequences are identical to those in the original transformation vector (pSYN12124)—no mutations
  —Except, the G event has a ~400 bp deletion in the promoter for crtl
- ►This deletion will require additional explanation and studies to characterize this unexpected occurrence
- ►1 000+ bp has been sequenced on each side of the inserts
  —G is located in an exon, R is in an intron and E is in an intergenic space
- ►All sequence/data reviewed by Humanitarian Board, Biosafety Resource Network, and Food Allergy Research & Resource Program(no issues other than those identified above)

(It will be clear that much of the decision making was again driven by the regulatory system, developed by signatories to the Cartagena Protocol. Despite the summary slide provided by IRRI, The Board had not reviewed, nor did most have the training, to 'review all sequence data' in any meaningful way, and it is unclear which other individuals had or the level of scrutiny afforded to it). The Board unanimously accepted the recommendation to change the lead event to GR2R.

In July 2010 a meeting involving the Director General of IRRI, and IRRI's Network Coordinator and Syngenta occurred at the companies head office in Basel to which neither the inventors nor the author

were invited (despite all three living very close by).

Just before the 15th Humanitarian Board meeting in Singapore on 26th and 27th of August 2010, IRRI's DG and the Golden Rice Network Coordinator of IRRI met with the inventors and the author and explained the contents of some late draft agreements resulting from the July 2010 meeting. One of the documents was a more complex form of Material Transfer Agreement than had been agreed in March 2009, for use in connection with the distribution of the GR2 lead transformation event to Golden Rice licensees.

It is unclear what thinking or which organisation prompted the July 2010 meeting in Switzerland. Later in 2010 Prof Beyer was informed by the Bill and Melinda Gates Foundation that the competitive grant he had been awarded by the Health Department of the Foundation in 2005 for line extension (eg improved second generation Golden Rice products) of Golden Rice would not be renewed at its termination in 2010. Instead the Foundation intended to award a grant for development of Golden Rice itself to IRRI, for management of Golden Rice out of IRRI.

The 16th Golden Rice Humanitarian Board meeting on 13th November 2011, was followed a day later by a Golden Rice Seminar at the CGIAR institute also in Washington DC the International Food Policy Research Institute (IFPRI), where IRRI announced the upcoming Gates Grant in support of Golden Rice, which of course was very welcome.

On 3rd October 2011 I gave an interview at the 10th Anniversary Meeting of the Conselho de Informacies Sobre Biotechnolgiain Sao Paolo Brazil. Amongst a lot of other commentary in a long interview I commented:"As of today, October, 2011, more than two and a half years on [from the March 2009 Lead Event decision], the selected Golden Rice seed has been supplied to research institutes in only two countries:India and Philippines. The inventors and the public sector Golden Rice licensees in other countries are very frustrated by this slow progress, at a time when multiple rice breeding programmes could be underway in multiple countries. All licensees already have the legal ownership of the Golden Rice trait. They need the physical materials. And use of the same physical materials—the same transformation event even in different varieties of rice—is effectively mandated by the regulatory environment. "

When finally published in August 2012[4], Dr Tang's research with Chinese children, initially spoken of in 2003/2004 showed that "The β-carotene in GR [Golden Rice] is as effective as pure β-carotene in oil and better than that in spinach at providing vitamin A to children. A bowl of ~100 to 150 g cooked GR [Golden Rice] (50 g dry weight) can provide ~60% of the Chinese Recommended Nutrient Intake of vitamin A for 6-8-y-old children. ""In summary, the high bioconversion efficiency of GR β-carotene to vitamin A shows that this rice can be used as a source of vitamin A. GR [Golden Rice] may be as useful as a source of preformed vitamin A from vitamin A capsules, eggs, or milk to overcome VAD in rice-consuming populations. Awareness of the vitamin A equivalence of plant foods provides a scientific basis for designing food-based nutritional programs to improve vitamin A status in many regions of the world where VAD is still common."

Twenty two days later, on 30th August 2012 Greenpeace issued a press release condemning use of a GMO-crop, Golden Rice, with Chinese children as 'guinea pigs of American researchers'. Actually, Dr Tang, and several of the other clinicians involved in the research were born and or are resident in China. Dr Tang, with 25 years' experience of similar research, and co-workers had previously conducted similar research with Golden Rice in USA with adults[22] and with children in China with other, non GMO-crop sources of beta-carotene[23]. Only Tang's 2012 research with gmo Golden Rice was criticised by Greenpeace.

As has been mentioned, more than a decade earlier in 2001, Greenpeace had also issued a Press Release in which it was claimed that Golden Rice could not be effective as an intervention against vitamin deficiency as an adult would have to eat at least twelve times the normal intake of 300 grams (eg 3.6 kilograms) of uncooked rice to obtain the daily recommended amount of pro-vitamin A. Clearly in 2012, in the light of Dr Tang results, Greenpeace were highly motivated to discredit her published results, but were unable to substantiate their 2012 allegations.

In 2012 and 2013 IRRI and the Philippines Rice Research Institute ('Phil Rice') planned and set out 5 multi-location field trials as part of the regulatory process for the Golden Rice containing transformation event GR2R. The approximate location of the trials was published on-line, in line with regulations, and also to comply with regulations for GMO-crop trials each location was surrounded by a high fence and patrolled by security guards day and night. On 8th August 2013 one of the (very easy to find) locations was vandalized by anti-GMO demonstrators.

The local authorities recognized that the demonstrators were not farmers, as was claimed and identified leading agitators from known organizations. The Philippine agricultural authorities undertook to track down and prosecute the individuals involved[24]. The destruction of the field trial was soundly condemned by the scientific community[25].

Despite the destruction of the one field trial, sufficient data was collected from the others to suggest that there was a yield drag compared to expected yield of the wild type rice variety. For any trait, especially a consumer trait such as nutritional enhancement, commercial growers expect excellent agronomy. (Government programmes, growing a crop to supply free to the nutritionally disadvantaged may chose different criteria). Normally commercial growers adopt new crop varieties and traits only because of increased profitability, and or ease of cultivation or processing both of which have economic benefits.

In a December 2013 meeting, the IRRI Network Coordinator appeared to recall the issue summarized in his December 2009 slide:"R is in an intron". Further investigation then suggested that the molecular data provided solely to IRRI in 2006, included information concerning both the intron insertion site, and (with only four computer mouse clicks by someone knowledgeable in the field) that the intron involved was Aux1, known since 1999 to be associated with root development (P Beyer, *pers comm*.).

On 15th May 2014 IRRI posted the following information concerning their Golden Rice research on their website: "The first round of MLTs (Multi Location Trials) was conducted using one of the most advanced versions of Golden Rice: GR2 event "R" (GR2-R). This first round took place in 2012-2013 to assess how well this version of Golden Rice would perform in different locations in the Philippines. Preliminary results were mixed. While the target level of beta-carotenein the grain was attained, average yield was unfortunately lower than that from comparable local varieties already preferred by farmers. An important goal of the trials was to test whether the agronomic performance of the new rice variety would be acceptable to farmers. The initial results indicate that more research is needed, with greater focus on increasing yield. Based on these results, a decision has been reached to move forward from work solely focused on GR2-R to also include other versions of Golden Rice, such as GR2-E and others. "…"IRRI and its many research partners remain committed to developing a high-performing Golden Rice variety that benefits farmers and consumers. The important mission of the Golden Rice project, i. e. , to contribute to improving the health of millions of people suffering from micronutrient deficiency, demands that every step and aspect of the scientific study of Golden Rice produces good results. IRRI and all participating organizations will continue to rigorously follow all biosafety and other regulatory protocols in continuing the research to develop and disseminate Golden Rice"[26].

5 What can Golden Rice's development history and trajectory teach us?

Progress from scientific vision, through research to proof of concept, through optimisation of technology, into seed breeding for a staple crop was always going to be challenging. Requiring new biosynthetic pathway engineering, new transformation capability and protocols for Asian rice varieties; for a new field:biofortified crops for micronutrient food security-especially so. And for such an economically, politically and religiously important crop as rice; and with a necessarily international programme across time zones and cultures; and across the normally distinct fields of agriculture and nutrition and sociology, particularly so.

Following the initial research success of the teams of Potrykus and Beyer, an innovative public private partnership amalgamated mutual efforts for differently defined objectives:public—not-for-profit humanitarian applications in developing countries and private—commercial exploitation as 'functional foods' in Europe and North America.

International cooperation was established with very high motivation and excellent communication from all people involved. Velocity was impressive as private sector crop scientists from Japan, Netherlands and UK and then the USA improved the potential for Golden Rice. Nutritional and clinical scientists from the US and China saw the possibilities and wanted to understand the potential. Funding came from the private and the public sector, governments and philanthropy. National, and the international, rice research institutes became involved in the project with enthusiasm to share skills and resources to develop Golden Rice in important locally adapted and preferred rice varieties. All involved understood the importance of achieving the objective of reducing unnecessary human misery for the hundreds of millions suffering from poor diet lacking sufficient source of vitamin A. All involved understood that poverty was the problem, that Golden

Rice couldn't cure poverty, yet could probably assist people to survive it and make better use of their opportunities while poverty itself was addressed by other means.

Right from the first Golden Rice Humanitarian Board meeting in August 2000, and at every subsequent step, scientific progress of research has been shaped and impeded by the regulatory requirements for GMO-crops. Gradually the restraining hand of these regulations developed by country signatories to comply with the hugely overcautious Cartagena Protocol[17], reduced the commercial attractiveness of the project for industrialised countries. Syngenta renounced commercial interest in 2004[20]. The ability of the international co-operators to share Golden Rice seed and pool their seed breeding resources, initially in 2001 accomplished almost as easily for Golden Rice to IRRI as for wheat seed varieties by Normal Borlaugh in the 1960's, was increasingly restricted as the malevolence of the Cartagena Protocol took root in increasingly bureaucratic obligations.

Political activism in the guise of health and environmental concerns took advantage of the suspicion of GMO-crop technology as a proxy for much of the activists discontent with globalisation. The pure vision of the Golden Rice Humanitarian Project became a 'must win' battle for the activists, for their ideology to prevail[5]. As the debate became more intense, some institutional participants became frightened of 'potential liability issues', further eroding willingness to share research materials and further impeding collaborative research and increasingly communication. Most international organisations quietly avoided any funding or association with 'GMO-crops' even those which had clearly huge potential for good, such as Golden Rice.

This impact of the Cartagena Protocol, and it's adoption as the basis of regulation by its many country signatories, is unfortunate:"The [precautionary] principle has long been a major impediment to good sense in public policy. It is either so obvious as to be otiose ("if there is cause for concern, be careful"), or so vague as to be meaningless. But in its most common application—"where an activity raises threats of harm to the environment or human health, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically"—it has been an invaluable tool for those who want to stop any new scientific development that they dislike"[27].

One of the most insidious anti-scientific impacts of the Cartagena Protocol derived regulations for GMO-crop research is preventing the skills of the plant breeder being able to select useful phenotypes of GMO-crop plants from early in the plant breeding process. This one restriction, unnecessary from any environment or human risk management perspective, delays the delivery of perfect crop varieties incorporating the trait of interest by many years, and increases the cost and complexity of the crops varieties development very significantly.

For all crop breeding, including GMO-crops, development has to involve the traditional skills of crop breeders and selection of plant phenotypes grown in the open field. Only growth of crops in the field, with all the biotic and abiotic stresses involved, allows the breeders to select useful plants, and discard the rest.

6 Conclusion

In 2004 UNICEF and the Micronutrient Initiative published a report at the World Economic Forum in Davos, Switzerland: Vitamin and Mineral Deficiency[28]. It acknowledged that "we are dealing with a global problem of enormous importance that is as yet little recognised". And that vitamin and mineral deficiencies 'debilitates the energies, intellects and economic prospects of 2 billion people and nations'. The report notes that in May 2002 the General Assembly of the UN called for the elimination of vitamin A deficiency by 2010. Vitamin A supplementation is not recommended for children younger than 6 months[1], and very young children do not consume solid food. Yet these children are the most vulnerable to vitamin A deficiency: neonate deaths in 2011 accounted for 43 percent (increased from 36 percent in 1990) of all deaths among under five-year-olds[29]. For breast milk to assist in the alleviation of vitamin A deficiency the mother must not herself be suffering from vitamin A deficiency:she must have adequate body stores of vitamin A. The beta-carotene in Golden Rice has been proven to have excellent bioavailability[4, 22] and "may

Fig.10 Golden Rice plants in the background, and around 100 g of polished Golden Rice grains in the Petri dish (The data suggests that about 40 grams of Golden Rice cooked and consumed daily will safely prevent blindness or death from vitamin A deficiency)

be as useful as a source of preformed vitamin A from vitamin A capsules, eggs, or milk to overcome VAD"[4].

For that half of the world's population where rice is the staple Golden Rice may have an important role in achieving the UN objectives laid out in the early 1990's and still not achieved 25 years later.

It is sad that current global society has to incur the human misery of blindness and death due to delays to advancement of Golden Rice caused by the regulations developed by national governments which are signatories to the UN's Convention on Biodiversity and its Cartagena Protocol, and human and institutional reactions to them. The ideas and concerns upon which the Cartagena Protocol is based were initially debated 50 years ago, and by now have been proved to have no merit. There is no risk from GMO-crops any greater than from crops bred using other technologies. Nevertheless, apart from the direct costs, the regulations feed suspicion of a useful and benign crop breeding development. It is for all these reasons that the Cartagena Protocol for Biosafety is inappropriate for GMO-crops and its effects should be nullified one way or another[17].

With respect to Golden Rice the costs of opposition to GMO-crops in India alone have been calculated at $200 million per year for the past decade[30]. Globally in 2010 vitamin A deficiency killed more children than either HIV/Aids, or TB or malaria[5]—somewhere around 2 million preventable deaths in that one year alone. That is 6 000 preventable deaths, mostly of young children, every single day. The 2004 UNICEF and Micronutrient Initiative report also says:"We have to leave behind old thinking and act in the light of new knowledge". In Golden Rice we have a simple and sustainable additional intervention for vitamin A deficiency with proven potential. The original vision is untrammelled. The "old thinking" we most have to leave behind is the UN's own Cartagena Protocol which is, without benefit, delaying its development.

With continuing patience, and subject to donors not giving up the funding and encouragement of institutional cooperation despite the political barriers erected to prevent it, the inventor's vision will be realised: sometime.

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